University of Alberta

Factors affecting the flight capacity and the trade-offs between flight and fitness in *Choristoneura conflictana* Walker (Lepidoptera: Tortricidae)

by

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Abstract

Energy expenditure for flight in insects may have consequences for subsequent survival and fecundity. *Choristoneura conflictana* Walker, a common defoliator of *Populus tremuloides* Michx. in Canada, were flown on computer-linked flight mills to establish how sex, mating status and age impact flight dispersal. Life history trade-offs between flight and fecundity, and flight and adult longevity were also examined. Females flew twice as far as males, and mated moths flew significantly farther than same-sex virgins. Moth age significantly affected distance flown by female, but not male moths. Female size and weight loss over the flight period affected the number of eggs laid by females, possibly due to egg resorption for energy use. Flight dispersal may be particularly important in the population dynamics of this cyclical forest species. Several factors shown here to influence individual flight and fitness will vary with population density under natural conditions.

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CHAPTER 1: GENERAL INTRODUCTION

Animal dispersal

Organisms move for a variety of reasons, ranging from small displacements for foraging to continent-spanning, multi-generational migrations as seasonal adaptations. There is a phase in the life cycle of every organism in which either long- or short-range dispersal takes place (Roff and Fairbairn 2001). Dispersal refers to the separation of individuals due to two types of intended movements, as well as unintended redistribution of individuals in a population (Woiwood *et al.* 2001; Dingle 2001). One type of intentional movement is displacement to obtain resources for survival and reproduction, and ceases when the resource is found. The second category of movement is migration in which organisms leave their colonized habitat to establish a new home range (Dingle 2001). Animals may disperse to find mates (Real 1990; Stamps 2001), to escape predation risks (Weisser 2001) or ephemeral habitats (Loxdale and Lushai 1999; Wiens 2001), or to locate resources. Theory predicts that animals will disperse to increase the benefits of survival and reproduction while keeping the costs of dispersal low (Stamps 2001). The study of dispersal is especially important with reference to population dynamics, because of the effect of habitat quality on growth, survival and reproduction of animals and their offspring (Loxdale and Lushai 1999; Ims and Hjermann 2001; Ishiguri and Shirai 2004; Benton *et al.* 2005) and the importance of mate choice in producing fit offspring (Wedell and Karlsson 2003).

Insect dispersal

In insects, populations disperse to forage (Loxdale and Lushai 1999; Dingle 2001), to breed (Dingle and Drake 2007), or to change habitats (Loxdale and Lushai 1999; Dingle 2001). Dispersal can occur in any mobile immature or adult stage. Most commonly, natal dispersal is facilitated by active movement or by wind (Torres-Vila *et al.* 1997) and adult dispersal by flight (Roff 1991; Dudley 2001; Roff and Fairbairn 2007) or small-scale movement in flightless individuals (Hardie *et al.* 2001). Such movement is difficult to study because it is affected by

many endogenous and exogenous factors, and occurs at many spatial scales (Johnson 1969; Loxdale and Lushai 1999; Schneider 1999; Muller-Landau *et al.* 2003).

Dispersal is adaptive due to temporal, morphological, or environmental factors that affect fitness, and some or all individuals in a population may disperse. Many insects have specialized migratory phases that are driven by environmental conditions that reliably cue dispersal at a certain point in the insect's life cycle (*Agrotis ipsilon* (Lepidoptera: Noctuidae) (Sappington and Showers 1992); *Melanoplus sanguinipes* (Orthoptera: Acrididae) (Min *et al.* 2004); *Danaus plexippus* (Lepidoptera: Nymphalidae) (Walton *et al.* 2005)). Morphological adaptations for dispersal include wing polymorphisms that result in distinct dispersal phenotypes in part of the population (Roff 1994; Langelletto and Denno 2001; Zhao and Zera 2002). In some species, females are flightless and males alate, which results in males assuming all dispersal costs as adults (Harrison 1994; Zera and Denno 1997). The suitability of the habitat impacts the likelihood of dispersal of individuals in a population, and can be driven by food availability, intraspecific competition (Gandon and Michalakis 2001), and overall habitat quality (Loxdale and Lushai 1999; Ims and Hjermann 2001). Physiological condition may also trigger dispersal of individuals. Coll and Yuval (2004) showed that small *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) have a greater propensity to fly than large, well fed individuals.

Due to the high energetic cost of flight (Dudley 2001), dispersal in insects often can result in trade-offs with other energetically costly processes, such as survival and reproduction (Boggs 1992). The concept of the oögenesis-flight syndrome (Johnson 1969) has been used in many studies to explain the energetic trade-off between reproduction and dispersal. However, this concept is controversial due to common misuse of the syndrome to explain trade-offs with different mechanisms (Sappington and Showers 1992). Females in some species however, have reduced fecundity (Gu *et al.* 2006) or egg size (Elkin and Reid 2005) as a result of energy expenditure on flight.

Dispersal capacity in insects can depend on insect sex, age, reproductive status, and size. Each of these factors, combined with environmental stimuli, influences the tendency and capability for insects to disperse. Flight differences associated with sex (Shirai *et al.* 1998; Hughes and Dorn 2002; Schumacher *et al.*1997; Senger *et al.* 2007) and age (Schumacher *et al.* 1997; Ishiguri and Shirai 2004) vary by species. These flight differences may depend on life cycle duration (Hughes and Dorn 2002) and larval and adult resource availability (Coll and Yuval 2004). Effects of mating status on dispersal vary by sex. In females, the high costs of reproduction (Yanagi and Miyatake 2003) change the resources available to be allocated to dispersal over the adult lifespan (Boggs 1992; Gu *et al.* 2006). Mated females may disperse after depositing some of their initial egg complement (Schumacher *et al.* 1997) to spread their progeny in suitable habitats (Loxdale and Lushai 1999). Virgin males may disperse to find a mate, especially at low population densities when females may be difficult to locate (Sanders 1983; Showers *et al.* 2001). Insect size affects dispersal in many ways, such as wing loading effects (Coll and Yuval 2004), and different energy reserves in differently-sized individuals (Shirai 1995; Zhao and Zera 2002). Population density strongly affects insect size and can indirectly and directly impact dispersal (Klemola *et al.* 2004; Hughes *et al.* 2001; Javoiš *et al.* 2005; Senger *et al.* 2007). Size also has an important impact on female fecundity (Honěk 1993; Jiménez-Pèrez and Wang 2004), which can affect dispersal in females that resorb eggs to allocate energy to flight or survival (Boggs and Ross 1993; Rosenheim *et al.* 2000).

Costs of dispersal

Dispersal to find a suitable mate, high quality habitat, and proper host for oviposition will occur if the benefits of dispersal outweigh the costs to an insect and their offspring (Ims and Hjermann 2001; Bowler and Benton 2005). The rate or likelihood of dispersal in a given species may depend on the fitness benefits of remaining in an inferior habitat against the energy expenditure of finding a more suitable location (Ims and Hjermann 2001). Dispersal distance is correlated with

the distance to this nearest suitable habitat, and selection for long-distance dispersal occurs when this habitat is far from an established population (Muller-Landau *et al.* 2003). Species that reside in temporary habitats or in ecosystems that undergo rapid changes are more likely to disperse (Ims and Hjermann 2001; Hanski *et al.* 2006). Once dispersal is initiated, the physical ability of the organism to deal with the costs of flight will influence the individual distance traveled (Roff and Fairbairn 2001; Yukilevich 2005).

A primary cost of dispersal is energy expended during flight, which includes flight muscle production and maintenance (Marden 2000) and energy to power the muscles (Sappington *et al.* 1995; Haunerland 1997). The time taken to search for resources, mates or suitable habitat, may delay mating and can reduce fecundity (Evenden *et al.* 2006). Insects use energy from lipids and carbohydrates in flight, depending on the species and type of flight (Judge *et al.* 1991; Sappington *et al.* 1995). It is difficult to measure the resources used in flight as analysis can only occur post-flight. Energy use during flight will likely differ not only among species but between individuals in a species, due to interactions between genotype and environment (Loxdale and Lushai 1999; Ims and Hjermann 2001).

Reduced longevity is another major cost of dispersal. Energy used in flight may reduce insect longevity in the same way that a lack of proper nutrition does (Elkin and Reid 2005). *Ceratitis* spp. (Diptera: Tephritidae) experience decreased longevity in response to poor nutrition (Manrakhan and Lux 2006). Shirai (1995) determined that larger *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) live longer and have a greater capacity for flight than small, shorter-lived moths. Mobile codling moths, *Cydia pomonella* L. (Lepidoptera: Tortricidae) are shorter-lived than moths that do not disperse (Gu *et al.* 2006). Since adult weight loss, more than absolute adult size, is thought to determine longevity (Leather and Burnand 1987), a measure of weight lost during flight is a good indicator of energy allocation and can indicate fitness trade-offs in the study system. Adult longevity may also be reduced in favour of increased reproductive

fitness (Yanagi and Miyatake 2003; Gu *et al.* 2006) or *vice versa* (Boggs and Ross 1993).

Insects commonly experience trade-offs between reproduction and dispersal. Female insects that expend energy on flight may produce fewer eggs (Elkin and Reid 2005; Gu *et al.* 2006) and can even reverse the flow of energy from reproduction to growth and survival by oöcyte resorption (Boggs and Ross 1993). Resources donated by males during reproduction (Boggs 1990; Smedley and Eisner 1996) may also be redistributed to power flight instead of increasing growth or survival of offspring. These studies indicate that there are finite resources available for flight, survival, growth and reproduction, and an increase in dispersal results in costs to other life history or reproductive strategies (Boggs 1992).

Population density

Population density is a dynamic state that can affect, either directly or indirectly, most other endogenous or exogenous factors that influence insect populations. Dense populations can alter habitat suitability by overexploitation of resources (Moreau *et al.* 2006) that may result in induced chemical defenses in plants (Clausen *et al.* 1989), or poor habitat quality (Loxdale and Lushai 1999). High insect densities may also reduce female fecundity and the fitness of subsequent generations (Awmack and Leather 2002; Klemola *et al.* 2004; Benton *et al.* 2005). In an insect with cyclical changes in population density, an initial density increase may occur as a result of large individuals with high fecundity (Myers and Kuken 1995). Herbivore population increase can also be facilitated by depressed defensive mechanisms in plants (Myers and Kuken 1995). However, once high population densities are reached, individual insect size may decrease due to the decrease in food availability (Klemola *et al.* 2004). Regardless of individual size, organisms that undergo outbreak cycles may increase their fitness by relocation to other habitats (Ims and Hjermann 2001).

Host quality can affect larval feeding and female oviposition. At high insect density the availability of nutrients can be reduced due to lack of suitable

foliage and reduced quality of the host (eg. *Epirrita autumnata* Borkhausen (Lepidoptera: Geometridae), Vertanen and Neuvonen 1999, Klemola *et al.* 2004; *Thaumetopoea pityocampa* Schiff. (Lepidoptera: Thaumetopoeidae), Hodar *et al.* 2002; Awmack and Leather 2002; *Choristoneura conflictana* (Walker) (Lepidoptera: Tortricidae), Clausen *et al.* 1989; *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae), Grant *et al.* 2007). Oviposition behaviour in some species, may reduce the density of offspring (*C. fumiferana,* Leyva *et al.* 2003) or optimize the use of oviposition substrate for larval development (*C. fumiferana,* Wallace 2004).

Benefits accrued from host selection will increase female dispersal (Loxdale and Lushai 1999; Coll and Yuval 2004) and may result in a decrease in the average size of individuals in a population (Klemola *et al.* 2004), especially if development time is altered in the new habitat (Klingenberg and Spence 1997). Insect size can alter longevity and fecundity (Torres-Vila *et al.* 2005) which demonstrates the importance of population density on the growth and survival of species. Forest Lepidoptera that experience cyclical population dynamics are excellent model systems to examine dispersal because of habitat and individual insect variation in these species. Especially well studied is the spruce budworm, *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae). *C. fumiferana* undergo extreme population cycling, and much is known about their mating habits (e.g. Outram 1971; Sanders and Lucuik 1992; Delisle and Hardy 1997), oviposition (e.g. Sanders and Lucuik 1975; Wallace 2004), and flight (e.g. Sanders *et al.* 1978; Greenbank *et al.* 1980; Sanders and Wilson 1990) under densely populated conditions.

Choristoneura conflictana

The genus *Choristoneura* contains many well-studied pest species in addition to *C. fumiferana*, including the orchard pest *Choristoneura rosaceana* (Harris) (Lepidoptera: Tortricidae)), as well as other outbreaking forest pests like *Choristoneura occidentalis* Freeman (Lepidoptera: Tortricidae) and *Choristoneura pinus pinus* (Freeman) (Lepidoptera: Tortricidae). Outbreaks of *C.*

occidentalis occur at population sizes 40% greater than endemic levels. Such large increases in population density are the result of climatic factors, heightened reproduction, and dispersal from nearby, high-density populations (Campbell 1993). In *C. pinus pinus*, outbreaks are associated with abiotic factors and larval host quality (Volney 1988).

Choristoneura conflictana (Walker) (Lepidoptera: Tortricidae) is a native defoliator that feeds primarily on trembling aspen, *Populus tremuloides* (Michx.) (Salicaceae) throughout its range in North America. *C. conflictana* are univoltine and overwinter as second instar larvae in diapause (Prentice 1955). The following spring, larvae emerge from hibernacula and feed on aspen buds, and then on fully-flushed leaves. After traversing 5 larval instars, *C. conflictana* pupate in a rolled leaf and eclose as adults ~1-2 weeks later, in mid-June in the boreal forest of western Canada (Prentice 1955). Eggs are laid in masses on the adaxial leaf surface and hatch in mid-July. First instar larvae feed until late August prior to winter diapause (Prentice 1955). Female *C. conflictana* continuously produce eggs over their adult lifespan. Females eclose with eggs in the ovaries, and lay more than one egg mass (Prentice 1955, Evenden *et al.* 2006). Adults live for 1-2 weeks, and feed to gather energy in addition to larval stores.

C. conflictana has 2-3 year population outbreak cycles, and is otherwise present at endemic levels in aspen-dominated ecosystems across Canada (Cerezke 1992). At high densities, *C. conflictana* will also feed on other hosts such as *Betula papyrifera* Marsh, *Salix* spp., and *Populus balsamifera* L. (Prentice 1955; Beckwith 1970). Like other outbreaking species, the cause of population increase of *C. conflictana* is largely unknown (Berryman 1996), but pathogens and parasitoids are thought to contribute to the decline of populations (Prentice 1955; Cerezke 1992). *P. tremuloides* responds to defoliation with an induced chemical defense that results in reduced *C. conflictana* survival (Clausen *et al.* 1989). Fitness and survival of *C. conflictana* are also impacted when larvae are forced to use secondary hosts (Beckwith 1970).

Objectives

Due to the increasing value of *P. tremuloides* in Canadian forests (Brandt *et al.* 2003) and the extreme and erratic population dynamics of *C. conflictana*, insight into this complex forest system will increase our understanding of population dynamics and defoliator impact on habitat. Defoliator dispersal capacity is a seldom studied factor that could greatly affect population dynamics of forest Lepidoptera. Here I assess mechanisms that may influence flight dispersal in adult *C. conflictana*. In Chapter 2, I assess the importance of sex, mating status, age, size, weight loss and body lipid content on the distance flown by *C. conflictana* using a flight mill system. In Chapter 3, I examine the impacts of dispersal on fitness correlates of longevity and fecundity to test the hypothesis that there is a trade-off between flight and reproduction in *C. conflictana*. Overall, I assess the impact that physiological parameters have on flight, and the consequences that high energy expenditure may have on the fecundity and therefore population dynamics of this cyclical forest lepidopteran.

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CHAPTER 2: Factors influencing the flight capacity of *Choristoneura conflictana* (Walker) (Lepidoptera: Tortricidae)

INTRODUCTION

Movement and dispersal by insects is influenced by many factors both at the individual and ecosystem level (Loxdale and Lushai 1999). The propensity and ability to disperse is dependent on the energy available to the insect and may be determined genetically (Honëk 1993; Wedell and Karlsson 2003; Yukilevich 2005; Gu *et al.* 2006) or by food quality and quantity throughout the insect's life (Boggs and Ross 1993; Coll and Yuval 2004; Gu *et al.* 2006). Endogenous factors including sex (Senger *et al.* 2007), mating status (Hughes and Dorn 2002), age (Hughes and Dorn 2002; Shirai 2006), size (Coll and Yuval 2004; Boggs and Freeman 2005) and lipid content (Judge *et al.* 1991; Coll and Yuval 2004) affect dispersal by flight in many insect species. Exogenous factors such as temperature (Hughes *et al.* 2001; Shirai 2006), habitat quality (Coll and Yuval 2004; Ishiguri and Shirai 2004) and population density (Doak 2000; Hughes *et al.* 2001; Nealis and Régnière 2004) influence flight dispersal by moths.

In insects, dispersal incorporates many levels of flight mobility, from foraging, to inter-habitat movement, to long-distance migration (Johnson 1969). Movement from one habitat to another may be prompted when the original location is unsuitable for optimal fitness of parent or offspring, and may be triggered by environmental conditions (Loxdale and Lushai 1999). Within the Lepidoptera, female and male moths disperse for different reasons. Male moths can fly long distances in search of a mate (Sanders 1983; Showers *et al.* 2001), and females disperse to find sites suitable for oviposition (Hughes and Dorn 2002). Female dispersal is therefore more likely to direct population movement or colonization efforts (Johnson 1966; Hughes and Dorn 2002; Benton *et al.* 2005), due to poor or dynamic conditions in the initial habitat (Loxdale and Lushai 1999; Ishiguri and Shirai 2004). Moth mating status (Hughes and Dorn 2002) and age (Schumacher *et al.* 1997a) can impact dispersal differently in males and females due to varying energetic investment at different life stages.

Movement to and from foraging sites and new habitats increases energetic demands for flying insects and can result in a resource trade-off between dispersal and reproductive output (Zhao and Zera 2002). Metabolism of lipids, carbohydrates, and amino acids provides energy to power flight muscles in insects, depending on the flight requirements (Judge *et al.* 1991; Sappington *et al.* 1995; Murata and Tojo 2002). Lipids are efficiently metabolized and are commonly used as an energy source by insects that disperse over great distances (Judge *et al.* 1991; Haunerland 1997). Lipid stores are gained through larval (Coll and Yuval 2004) or adult feeding (Wheeler 1996; Hanski *et al.* 2006) and remain in the fat body until hormonal stimulation induces transfer to the hemolymph, or directly to the flight muscle (Haunerland 1997).

High density populations of *Choristoneura conflictana*, that result in severe local defoliation of *Populus tremuloides* may initiate female dispersal to a new site, or extend the original population epicentre outward. Outbreaks of *C. conflictana* populations can result in defoliation over 25,000 km² (Prentice 1955). Herbivory caused by high density populations of *C. conflictana* induces the production of chemical defenses in *P. tremuloides* and reduces host suitability for larvae (Clausen *et al.* 1989) and oviposition habitat for females. Use of host species other than *P. tremuloides* reduces survival, size and female fecundity in *C. conflictana* (Beckwith 1970). Body size and fecundity are positively correlated with population density in some forest Lepidoptera (Myers and Kuken 1995), whereas in other species the correlation is negative (Klemola *et al.* 2004; Nealis and Régnière 2004). Body size affects wing loading during flight as well as the lipid stores available to power flight and can directly influence flight capability (Coll and Yuval 2004). Other *Choristoneura* species are deterred from ovipositing on non-host plants (Grant and Langevin 1995) or in areas with many conspecific egg masses (Leyva *et al.* 2003) making dispersal a likely consequence of female-saturated habitats.

The capacity for insects to disperse has been studied using various methods, including mark-recapture (e.g. Sanders 1983; Schneider 1999). This

technique allows for a field-based assessment of distance flown through insect trapping, but requires large numbers of insects for release, and destructive sampling can result in cessation of flight short of an insect's true flight capacity. Laboratory-based assessments of flight dispersal such as wind tunnel (eg. Sanders 1985) or flight mill bioassays (eg. Mason *et al.* 1989; Schumacher *et al.* 1997a and b) can give an accurate measure of distance flown under controlled environmental conditions. However, dispersal capacity measured in the laboratory will not be directly applicable to potential dispersal in the field. In this study, I use a rigid-arm, computer-linked flight mill to directly compare distances flown by *C. conflictana* in different treatment groups. A comparison of energy use in natural *versus* tethered flight on *Cicadulina storeyi* (Homoptera: Cicadellidae) indicates that flight on flight mills requires at least 20-30% more energy than free flight due to friction on the mill axis (Riley *et al.* 1997). Thus, the distance flown on flight mills cannot be directly transferred to flight in natural systems, but provides a relative estimate of flight capacity among treatment groups (Schumacher *et al.* 1997b) that can assist when making hypotheses about natural dispersal.

In this chapter, I use computer-linked flight mills to test the effect of several parameters predicted to influence flight dispersal of *C. conflictana*. Knowledge about the factors that affect dispersal will give insight into the potential importance of movement in the population dynamics of this important defoliator. Due to the high energetic costs of flight, I hypothesize that flight dispersal by *C. conflictana* varies by sex, age, mating status, size and body lipid content. In the forest, *C. conflictana* flight will also be affected by population density and climatic factors. However, assessment of flight capacity in a controlled environment allows me to determine which factors may be driving variation in dispersal in this cyclical forest pest.

METHODS

INSECT COLLECTION AND REARING

Choristoneura conflictana (Walker) were collected as eggs and larvae in Rocky Mountain House (52.4° N, 114.9° W) and Drayton Valley (53.2° N, 115.0° W), Alberta in the first week of June, 2004. Insects used in flight mill bioassays were reared in the laboratory and were one or two generations from collection. First instar larvae were fed a wheat-germ based, synthetic spruce budworm diet (Bio-Serv, Frenchtown, NJ, Appendix I) after egg hatch. Second instar larvae were provided with a 16 cm^2 cheesecloth square as an overwintering site held at 2-4°C in total darkness for twenty weeks. They were placed at room temperature for emergence after removal from overwintering conditions. Each larva was removed from cheesecloth with an artist's No. 1 paintbrush (Colours Classic), and placed individually into a 30 ml cup on ~10 ml of fresh synthetic diet and held at 23°C under a 16:8 light:dark photoregime for the remainder of larval development. Pupae were sexed and individually transferred to a labelled 150 ml translucent cup and held under rearing conditions until adult eclosion. Adult moths were supplied with 10% sugar water *ad libitum* through a cotton wick.

FLIGHT BIOASSAYS

Apparatus

Fixed-arm (30-cm long) computer-linked flight mills were used to measure flight capacity of *C. conflictana* (Figure 2.1A). Each rotation of the mill arm was registered by a small infra-red transmitter/receiver situated on the mill casing (Figure 2.1B) that sent a signal to the attached computer (MaxDigital, 250MB). A computer program (Flysumm Data, 2000 and 2006) written especially for use with these mills, recorded each revolution (94 cm) as moths propelled the arm. Mills were housed in a walk-in growth chamber held at 23 °C under a reversed 16:8 light:dark photoregime to allow flights in the scotophase to be initiated during the day. Up to eight mills were run simultaneously on each day of experimentation depending on moth availability.

Protocol

Moths collected from rearing conditions were cooled for 5 minutes at 4°C and then weighed to the nearest 0.01 mg (Mettler Toledo, XS105, Columbus, OH). Individual moths were then harnessed at room temperature. To harness moths, each individual was held with soft forceps and scales were carefully brushed off the pronotum using a modified artist's No. 1 paintbrush with cut bristles. A piece of 1 mm (diam.) aluminum wire with a 2 mm (diam.) loop at one end and a 2 cm straight portion of wire perpendicular to the loop was used to tether each moth (Figure 2.1C). The loop was dipped in Lepage's ® Pres-Tite Contact Cement (Brampton, ON) and pressed flat to the scale-free pronotum for 20-30 seconds until it adhered. Once 8 moths were harnessed, they were transferred to the flight mill room and each harness was attached to the distal end of a flight mill arm using a 3 cm length of wire insulation (2 mm diam.) at ~100° with the mill arm. If necessary, each individual was given one or two tarsal touches with a clean glass slide to stimulate flight. Moths were then left undisturbed for the remainder of the flight period. Flight bioassays were 12 hours in length, began 0.5 hours prior to the onset of scotophase (5 lux), and continued until 3.5 hours into the photophase (268 lux). After flight, each moth was weighed again and returned to a 150 ml cup. The right forewing was removed from each moth and glued to a paper-covered piece of cardboard. Each piece of cardboard was scanned (Hewlett-Packard Scanjet 4070 Photosmart, Mississauga, ON) to obtain a digital image of each wing. The wing area of each wing was measured using ImageJ software (Version 1.37, 2006).

Lipid extraction

Body lipid content at the end of each bioassay was measured using a Soxhlet apparatus (Fisher Scientific, 45/50 Pyrex, Ottawa, ON). Appendages were removed and each moth was dried for 24 hours in a 75°C drying oven (Cenco Instruments, 1200 watts, Chicago, IL). Desiccated moths were weighed and placed individually into perforated 0.5 ml microcentrifuge tubes. Twenty-five tubes were placed in the soxhlet apparatus and the lipid was extracted over 6

hours with petroleum ether (Fisher Chemicals, E139-4) as the solvent (Elkin and Reid 2005). After the extraction, insects were dried again for 24 hours at 75°C. To determine the percent lipid content of each moth at the end of the bioassay the following formula was used:

$$\frac{\text{(Pre-extraction dry weight)} - \text{(Post-extraction dry weight)}}{\text{Pre-extraction dry weight}} \times 100$$

EXPERIMENTS

Effect of sex on flight

In Experiment 1, I tested the statistical hypothesis that virgin male and female moths differed in their capacity to fly. On each of 14 days, a set of 8 males aged 2-4 days old or 8 females aged 1-3 days old were prepared for flight 1 h prior to the onset of the scotophase. Males and females were flown on separate days so that any potential pheromone release by females did not alter male flight. The flights of 53 males and 49 females were compared.

Effect of mating status on flight

Two experiments were conducted to test the hypothesis that flight capacity differed between virgin and mated females (Experiment 2) and males (Experiment 3). In Experiment 2, 8, 0-day old (<24 h post-eclosion) females were removed from rearing conditions 1 h prior to the onset of scotophase. Females were individually placed in wax-lined, cardboard, 473 ml Solo ® cups and provided 10% sugar water. Four of the females were paired with a 2-3 day old male, and the other 4 with a second female to ensure similar handling procedures in both treatment groups for a 24 h period. Mated and virgin females were flown on flight mills during the following scotophase at 1 day post eclosion. A total of 52 virgin and 55 mated females were flown over 25 days. Females that had been paired with a male were dissected at the end of the bioassay to verify their mating status. Females with a spermatophore in the *bursa copulatrix* were scored as mated. In Experiment 3, 1-day old males were paired with a receptive virgin female or another male for 24 h. Virgin and mated males were flown during the following scotophase at 2 days post eclosion. A total of 10 mated and 28 unmated

males were flown over 20 days. Males were dissected to ensure their mating status (Evenden *et al.* 2003).

Effect of age on flight

I tested the hypotheses that moth age would affect flight capacity in female (Experiment 4) and male (Experiment 5) *C. conflictana*. In each experiment, virgin moths of differing ages (0, 2, 4, and 6-days old) were flown on the same day. In Experiment 4, ~30 females in each age category were flown over 26 days. In Experiment 5, ~30 males in each age category were flown over 28 days.

Effect of lipid content

Moths from all experiments (with the exception of Experiment 5 in which the males were dissected to determine mating status) were pooled to assess the effect of flight, mating status, age and size on body lipid content. In this case, percent body lipid was treated as the dependent variable to explain flight capacity instead of an independent variable as in Experiments 1-4.

ANALYSES

For each experiment, a separate stepwise general linear model was conducted (SYSTAT®12, 2007). In Experiments 1-5 flight distance was the dependent variable. Flight distance data were log + 1 transformed to improve normality (Kolmogorov-Smirnov test, SPSS 11.5, 2006). In analyses of all experiments, day of flight was included as an independent variable to account for any variation in conditions among flight days. In Experiment 1, sex was treated as the main independent factor and adult pre-flight weight, weight lost over the flight period, and percent lipid content were included in the model as covariate factors. Interactions between the main factor and each of the covariates were also included in the GLM. In Experiments 2 and 3, mating status (virgin or mated) was treated as the main effect in the model, and adult pre-flight weight, weight loss and lipid content were considered as covariates. Interactions between each of the covariates and mating status were also included in each model. Non-significant factors were removed from the model automatically in a stepwise

manner until the most parsimonious model remained (SYSTAT® 12, 2007). The effect of age on flight distance in Experiments 4 and 5 was analyzed with age specified as the main effect followed by Tukey HSD to compare the individual age effects (SYSTAT®12, 2007). Covariates included in the model were adult pre-flight weight, weight loss during flight, lipid content and interactions of all covariates with the main effect of age in each experiment.

A stepwise general linear model was also tested with lipid content as the dependent variable on the pooled data from Experiments 1-4, to assess the effect of sex, mating status and flight status as main independent effects on resulting percent body lipid content. Sex, mating status and flight status and all possible 2 and 3-way interactions were tested in the model. Pupal weight was incorporated into the model as a covariate to account for variation in body size (Appendix II) and interactions between pupal weight and each main effect were included in the model.

RESULTS

Effect of Sex

In Experiment 1, there was a significant interaction between sex and percent body lipid content on distance flown. Virgin female *C. conflictana* flew farther than virgin males (Stepwise GLM, $r^2 = 0.13$, $F_{1,91} = 5.031$, $p_{sex*lipid\ content} = 0.027$) (Figure 2.2). Females flew an average of 1.6 km (+/- 160.5 SE), and the longest recorded distance by one female was 26.5 km in a 12 hour period. The average flight distance for males was 283 m (+/- 41.6 SE), and the maximum flight distance was 5.2 km. The significant interaction between lipid content and total distance flown showed that males that flew farther subsequently had lower lipid levels than short-distance fliers. In contrast, females that flew farther had higher lipid levels than females that flew short distances.

Effect of mating status

Mated females flew significantly farther than virgin females in Experiment 2 (Stepwise GLM, $r^2 = 0.27$, $F_{1,86} = 13.45$, $p_{mating\ status} < 0.0005$) (Figure

2.3) and heavier females flew farther than lighter females ($F_{1,86} = 28.25$, $p_{preflight\ weight} < 0.0005$).

Mating itself did not have a significant effect on the flight of *C. conflictana* males in Experiment 3, but there were significant interaction effects between mating status and weight loss during flight (Stepwise GLM, $r^2 = 0.18$, $F_{1,36} = 7.60$, $p_{mating\ status*weight\ loss} = 0.009$) and between mating status and pre-flight weight ($F_{1,36} = 6.63$, $p_{mating\ status*pre\text{-}flight\ weight} = 0.014$) on the distance flown by males. When weight loss and pre-flight weight were interacted with mating status in the model, mated males flew farther than unmated males (Figure 2.4).

Effect of Age

Age significantly determined the total distance flown by female *C. conflictana* in Experiment 4 (Stepwise GLM, $r^2 = 0.30$, $F_{3,144} = 3.26$, $p_{age} = 0.023$) (Figure 2.5) and there was a significant interaction effect between age and lipid content ($F_{3,144} = 3.17$, $p_{age*lipid\ content} = 0.026$). Pre-flight weight also significantly contributed to prediction of distances flown by females in Experiment 4 ($F_{1,144} = 22.10$ $p_{weight} < 0.0005$). This indicates that both size and body lipid content promote dispersal in female *C. conflictana*. The general trend was that older females fly farther, but the differences in average distance flown were significant between 0 and 2 day old females (Tukey's HSD, $p = 0.015$), 0 and 6 day old females (Tukey's HSD, $p < 0.0005$) and 4 and 6 day old females (Tukey's HSD, $p = 0.027$).

In contrast, age was not a significant factor in determining distance flown by male *C. conflictana* in Experiment 5, although a trend towards older males flying farther was evident (Stepwise GLM, $r^2 = 0.11$, $F_{3,120} = 2.35$, $p_{age} = 0.076$) (Figure 2.6). Weight lost by male moths over the flight period significantly affected distance flown ($F_{1,120} = 3.57$, $p_{weight\ loss} = 0.014$). Interestingly, males that flew short distances lost more weight than males that flew long distances.

Lipid content

When lipid content was treated as the dependent variable, sex (Stepwise GLM, $r^2 = 0.20$, $F_{1,821} = 39.04$, $p_{sex} < 0.0005$), flight ($F_{1,821} = 8.56$, $p_{flown} = 0.004$)

(Figure 2.7), and mating status ($F_{1,821} = 8.97$, $p_{mating\ status} = 0.003$) were all significant main effects in determining body lipid content of *C. conflictana*. Males had a higher percentage body mass of lipid than females at the time of measurement. Surprisingly, flown moths had a greater percentage of body lipid content than unflown moths (Figure 2.7). Interaction between flight and pupal weight was also significant ($F_{1,821} = 8.55$, $p_{flight*pupal\ weight} = 0.004$). Mated moths had a higher lipid content than virgins. The interaction between mating status and pupal weight ($F_{1,821} = 4.55$, $p_{mating\ status*pupal\ weight} = 0.033$) and between sex, mating status and pupal weight ($F_{1,821} = 7.11$, $p_{sex*status*pupal\ weight} = 0.008$) were also significant (Figure 2.8).

DISCUSSION

Sex

Sex itself did not significantly affect flight distance of *C. conflictana*, but there was a significant sex by lipid content interaction term that impacted total distance flown. As would be predicted, males that flew short distances had an higher lipid content than males that flew long distances. In contrast, females that dispersed long distances had higher lipid reserves than short distance fliers. This may be because females can reverse the allocation of energy from reproduction back to survival, through egg resorption (Boggs and Ross 1993; Rosenheim *et al.* 2000), therefore gaining additional resources for flight.

When lipid content was considered, female *C. conflictana* flew farther than males. *C. conflictana* females have fully developed eggs upon adult eclosion that could be resorbed during flight. Boggs and Ross (1993) found that when under nutrient stress, females of *Speyeria mormonia* Boisduval (Lepidoptera: Nymphalidae) increased survival by resorbing their eggs. If energy from egg resorption is used in females to conserve lipid stores for survival, this may explain why female *C. conflictana* that flew long distances, retained more lipid than short-flyers. Alternatively, flight may be less energetically expensive in females than in males because of their greater wingspan and reduced wing loading (Coll and Yuval 2004). In other flight mill studies, female *Grapholita molesta* (Busck)

(Lepidoptera: Tortricidae) (Hughes and Dorn 2002) and *Homona magnanima* Diakonoff (Lepidoptera: Tortricidae) (Shirai *et al.* 1998) flew farther than conspecific males. However, male *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) (Schumacher *et al.* 1997a) and *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae) (Gu and Danthanarayana 1990) are capable of flying longer distances than conspecific females. In these tortricids, sexual size dimorphism, and differences in wing loading, may not be the crucial factor in the determination of dispersal capability.

Moth condition differentially affects dispersal of male and female *C. conflictana*. The distance flown by *C. conflictana* was significantly affected by the interaction between sex and body lipid content. Male *Choristoneura fumiferana* (Clemens) flight dispersal can be related to moth condition at different population densities (Sanders 1983). In tephritid flies, male and female flight response differs with population density, although the average flight by both sexes was similar (Senger *et al.* 2007). Since moths on the flight mills in my study would never encounter a mate or a host, average dispersal distance may have been overestimated. Field studies with external cues that can affect flight distance are required to determine if both endogenous and exogenous factors interact to affect male and female *C. conflictana* dispersal.

Mating status

Mated females of *C. conflictana* flew farther than virgin females. This suggests that either mating induces dispersal in females; or mated females, due to oviposition of a portion of their egg load after copulation, are better able to fly (Schumacher *et al.* 1997a). Female dispersal to oviposition sites may be particularly important for species that experience large fluctuations in population density. In females of *Carposina sasakii* Matsumura (Lepidoptera: Carposinidae) dispersal is induced at high population densities and female flight capability remains high after mating (Ishiguri and Shirai 2004). In *C. pomonella*, although mated females disperse farther than virgins, the ability to fly decreased faster in mated females because of the lipid cost of reproduction (Schumacher *et al.*

1997a). In species that do not experience cyclical population dynamics, mated and virgin females fly similar distances (Shirai *et al.* 1998; Shirai and Kosugi 2000; Hughes and Dorn 2002; Ishiguri and Shirai 2004; Shirai 2006). At high population densities, *C. conflictana* (Prentice 1955; Cerezke 1992) may experience low-quality oviposition habitat in a defoliated area, and therefore respond to environmental cues to disperse (Senger *et al.* 2007). Increased dispersal by mated female *E. postvittana* and *C. pomonella* is attributed to increasing the breadth of oviposition locations (Gu and Danthanarayana 1990; Schumacher *et al.* 1997a).

Mating status was not a significant main effect for male flight distance, but mating status*weight lost and mating status*pre-flight weight did significantly affect the distance flown by male moths. When weight loss and initial size were taken into account, mated males flew farther than virgin males, which indicates size, energy expenditure, and flight efficiency are important determinants of male flight capacity. Hughes and Dorn (2002) suggest that close proximity of females at the eclosion site promotes short flights in virgin, male *G. molesta* and longer flights in mated males. There may also be a reduced fitness cost to dispersal of mated males (Hughes and Dorn 2002). If female *C. conflictana* are abundant at high population densities, mate finding by virgin males would occur over short distances. Males may be stimulated to fly longer distances to find a second mate, but may improve their fitness by initially mating early as delays in mating reduce reproductive output in female *C. conflictana* (Evenden *et al.* 2006).

Age

Age and pre-flight weight of female *C. conflictana* both significantly influence flight distance, and there is a significant interaction between age and body lipid content. In general, older and heavier females fly farther. Female flight distance also varies with age in *G. molesta* (Hughes and Dorn 2002). In these tortricids, flight distance increased up to age 3 days and then decreased after 5 days (Hughes and Dorn 2002). *C. conflictana* are generally shorter-lived than

G. molesta, so older moths could not be compared. Older *C. conflictana* females may have flown even farther, as they deposit unfertilized eggs at ~ 4 days old (data not shown). Oviposition would decrease the wing loading of the 4 and 6-day old females tested here (Coll and Yuval 2004; Schumacher *et al.* 1997a). Heavier females may fly farther due to greater stores of resources including lipid (Canavoso *et al.* 2001), or due to egg resorption to reverse energy flow (Boggs and Ross 1993; Rosenheim *et al.* 2000). Roff (1991) predicted that migrating insects would benefit more from being heavy than sedentary insects, due to the high energetic cost of active movement. Interestingly, differently aged female *C. sasakii* fly similar distances, but moth mortality increases significantly over the flight period as moths get older, which implies increased impact of flight on the survival of aging females.

Age did not significantly affect the distance flown by males of *C. conflictana*. Surprisingly, males that fly longer distances lose less weight over the flight period than those that cover short distances. This may be because strong fliers have a greater proportion of their body weight made up of flight muscle which is highly correlated with flight performance (Marden 2000). Male age also does not affect flight capacity in *G. molesta* (Hughes and Dorn 2002). However, there is a decline in male flight capacity as *C. pomonella* age (Schumacher *et al.*1997a). Flight muscle may begin to degenerate over the longer lifespan (~21 days) of *C. pomonella* compared to the relatively young (<6 day) *C. conflictana* tested in this study.

Lipid content

Males of *C. conflictana* have a greater percentage body lipid content than females, although they are smaller and weigh less than females. Although mature oocytes have ~40% lipid content (Canavoso *et al.* 2001) lipid extraction was done post-oviposition in most females in my studies. Males and females may also respond to resource shortages by metabolizing lipid stores differently under natural conditions (Senger *et al.* 2007). There is a physiological difference in the use of lipid *versus* carbohydrate energy sources in male and female insects (Judge

et al. 1991). In the migratory moth species, *Agrotis ipsilon* Hufnagel (Lepidoptera: Noctuidae), 'long-flyers' depend more on carbohydrates than 'short-flyers' (Sappington *et al.* 1995). Thus, variation in flight and the type of energy mobilized and allocated to flight appears to be mediated by both genetic and environmental factors. Interestingly, female moths flown in my study had higher lipid content than those that did not fly. In noctuids, lipids are the primary energy source metabolized while resting (Sappington *et al.* 1995). If this is also the case in *C. conflictana*, individuals left at rest may utilize more lipid stores than those in flight, that may rely more heavily on carbohydrates (Sappington *et al.* 1995). Mated female *C. conflictana* have higher lipid content than virgin females, likely because mating induces further egg production (Outram 1971; Adams 2000) and mature eggs that remain in the ovaries are rich in lipid (Canavoso *et al.* 2001).

Flight capability in the aspen defoliator *C. conflictana* appears to be affected by endogenous as well as exogenous factors. Much of their flight activity is highly dependent on body size and body lipid content, both factors that are likely to vary greatly with population density. In nature, both of these factors are mediated by habitat quality and available resources. The idea that females disperse to oviposit in a more suitable habitat in a non-migratory species such as *C. conflictana* is both supported and refuted by other studies in the literature, but evidence from the current study is favourable. Females in species that undergo population cycles are less specific when choosing their oviposition host, and this contributes to their ability to attain high population densities (Price 1994). However, in other lepidopteran species flight capacity remains high after mating (Gu and Darantharayana 1990; Schumacher *et al.* 1997a; Ishiguri and Shirai 2004) which suggests that moths disperse to oviposit. How these findings relate to dispersal of *C. conflictana* movement in nature remains to be seen but further questions might address the response of *C. conflictana* to different levels of nutrition or bioassays that better reflect natural flight without time or space limitations.

(A)

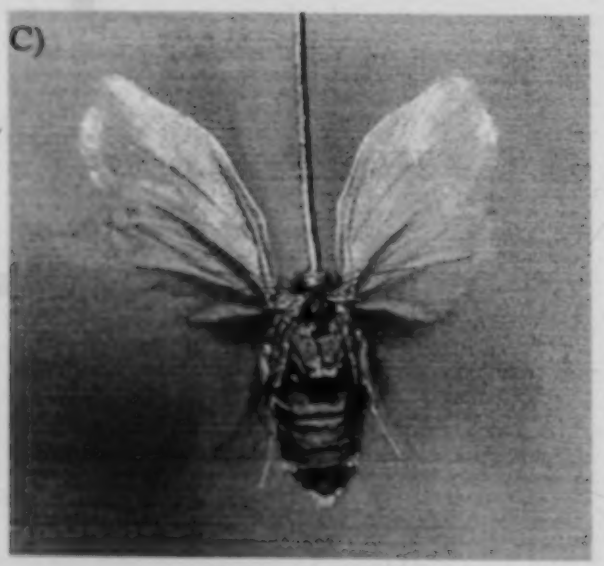

Figure 2.1. A) Flight mill apparatus with a 30 cm rigid arm that rotates on a magnetic axis. Red wire insulation at the terminal end of the arm is the place of insect attachment. B) A closer view of the sensory apparatus beside the flight mill axis. As the round, reflective disc passes the black sensor, a single rotation of 94 cm is recorded on the attached computer. C) A female *C. conflictana* on flight harness.

Figure 2.2. The average distance flown in meters by male and female *C. conflictana* over a period of 12 hours, measured on computer-linked flight mills in Experiment 1. Bars represent standard error, $n_{female} = 49$, $n_{male} = 53$. Mean values for distance flown are adjusted to account for the covariate effects in the model. Females flew significantly farther than males when percent body lipid content was considered in the model (Stepwise GLM, $r^2 = 0.13$, $F_{1,91} = 5.031$, $p_{sex*lipid\ content} = 0.027$). Different letters represent statistical significance ($p < 0.05$).

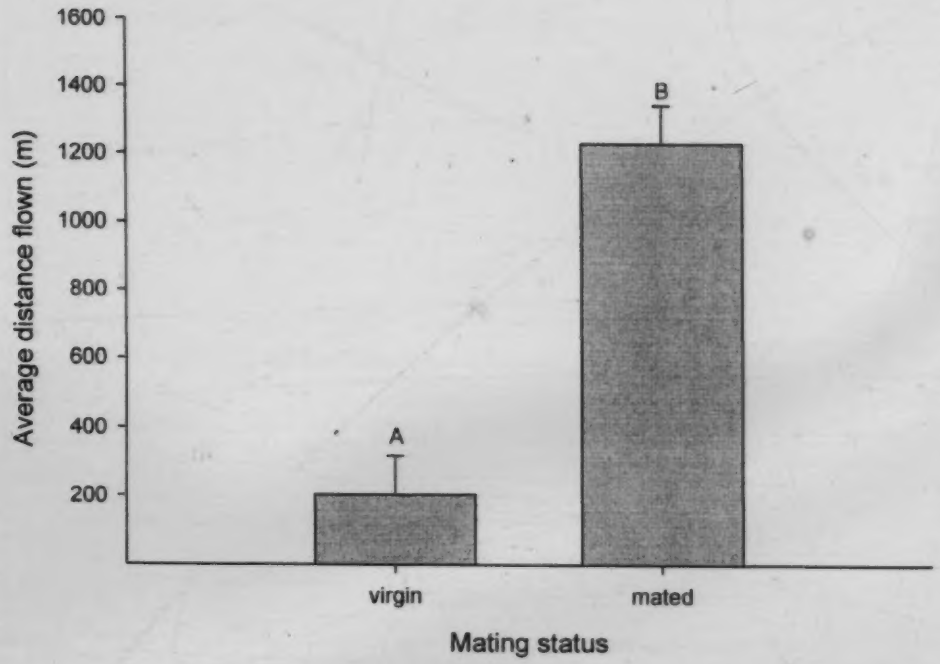

Figure 2.3. The average distance flown in meters by mated and virgin female *C. conflictana* over a period of 12 hours, measured on computer-linked flight mills. Bars represent standard error, n = 45 per treatment. Distance flown values are adjusted to account for the covariate effects. Mated females flew significantly farther than virgins (Stepwise GLM, $r^2 - 0.27$, $F_{1,86} = 13.45$, $p_{\text{mating status}} < 0.0005$). Different letters represent statistical significance ($p < 0.05$).

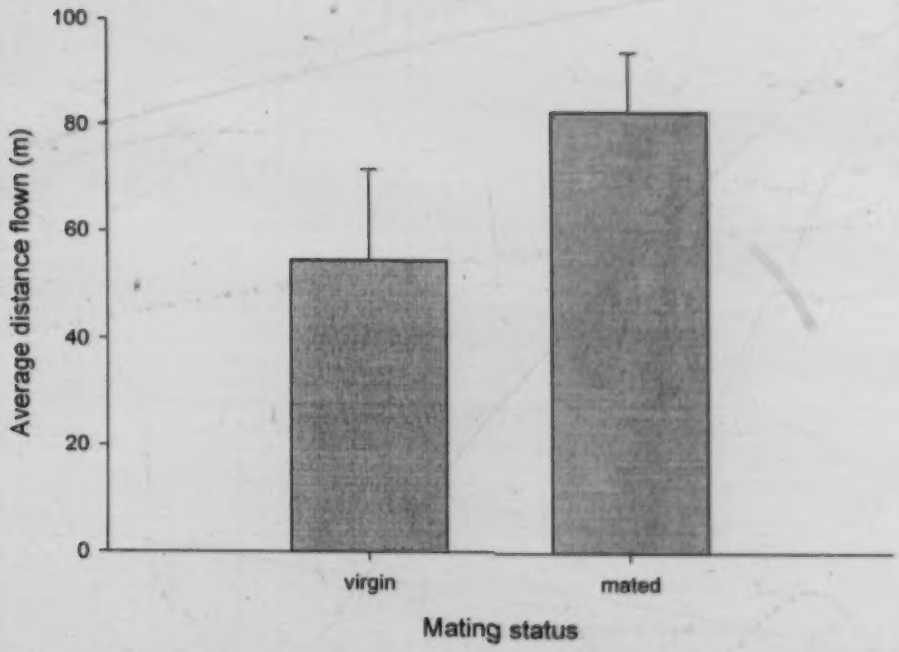

Figure 2.4. The average distance flown in meters by mated and virgin male *C. conflictana* over a period of 12 hours, measured on computer-linked flight mills in Experiment 3. Bars represent standard error, $n_{virgin} = 28$, $n_{mated} = 8$. Distance flown values are adjusted to account for covariate effects. Mated males flew significantly farther than virgins when weight loss and pre-flight weight were taken into account (Stepwise GLM, $r^2 = 0.18$, $F_{1,36} = 7.60$, $p_{mating\ status*weight\ loss} = 0.009$), ($F_{1,36} = 6.63$, $p_{mating\ status*pre\text{-}flight\ weight} = 0.014$).

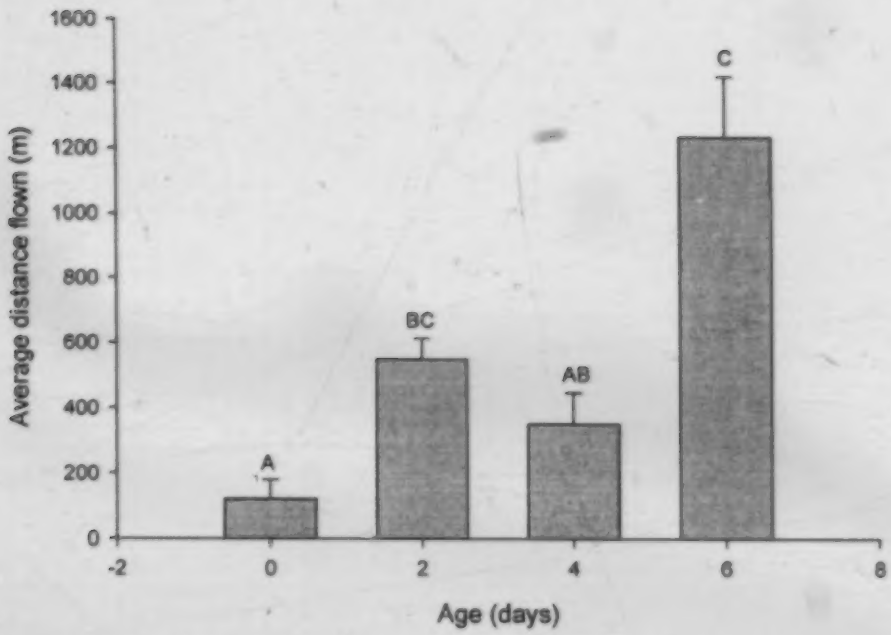

Figure 2.5. The average distance flown in meters by female *C. conflictana* aged 0, 2, 4, and 6 days post eclosion over a 12 hour period, measured on computer-linked flight mills in Experiment 4. Bars represent standard error, $n_0 = 40$, $n_2 = 37$, $n_4=42$, $n_6=39$. Distance flown values are adjusted to account for the covariate effects (Stepwise GLM, $r^2 = 0.30$, $F_{3,144} = 3.26$, $p_{age} = 0.023$). Different letters represent statistical difference of the means ($p < 0.05$), as determined by a post-hoc Tukey's HSD test.

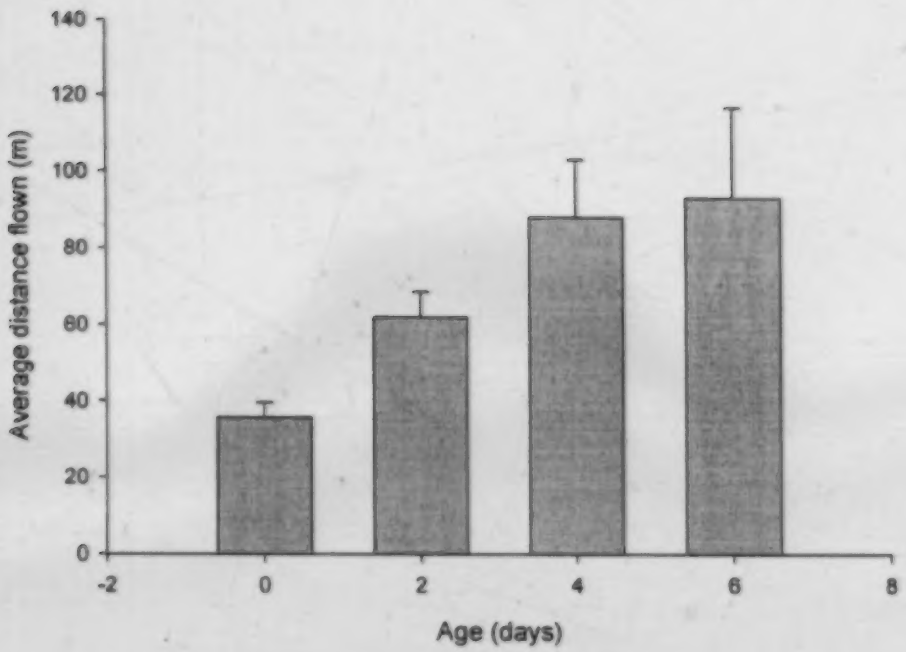

Figure 2.6. The average distance flown in meters by male *C. conflictana* aged 0, 2, 4, and 6 days over a 12-hour period, measured on computer-linked flight mills. Bars represent standard error, n_0=33, n_2=37, n_4=35, n_6=23. Distance flown values are adjusted to account for the covariate effects. There was no statistical difference in distance flown among ages tested (Stepwise GLM, $r^2 = 0.11$, $F_{3,120} = 2.35$, $p_{age} = 0.076$).

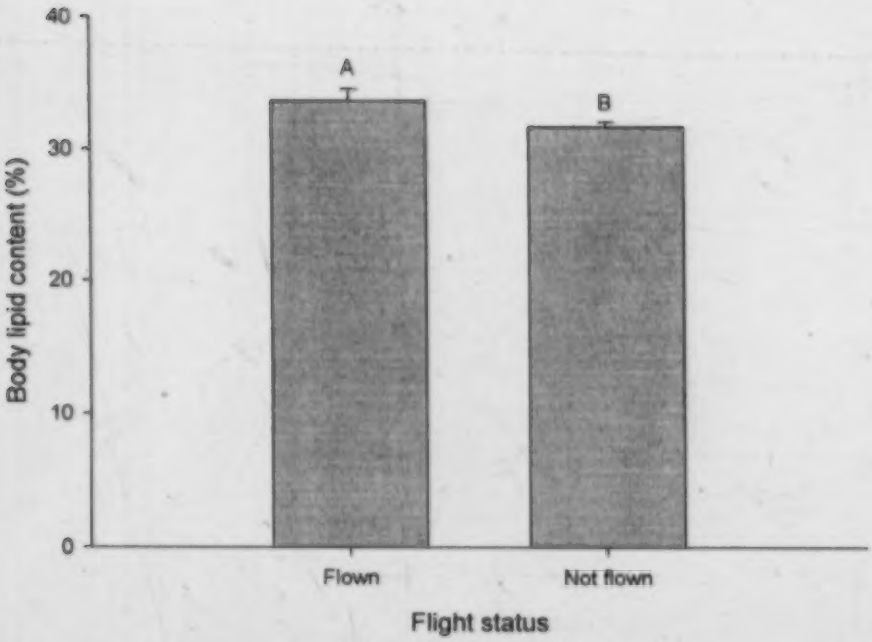

Figure 2.7. The percent moth body weight composed of lipid in *C. conflictana* from Experiments 1-5. Flown individuals flew for 12 hours on computer-linked flight mills, and individuals that did not fly remained in rearing cups for that period. Lipid content was measured using a soxhlet apparatus after moths died. Bars represent standard error, n_{flown}=486, $n_{not\ flown}$=344. Lipid content values are adjusted to account for the covariate effects. (Stepwise GLM, $r^2 = 0.20$, $F_{1,821} = 8.56$, $p_{flown} = 0.004$).

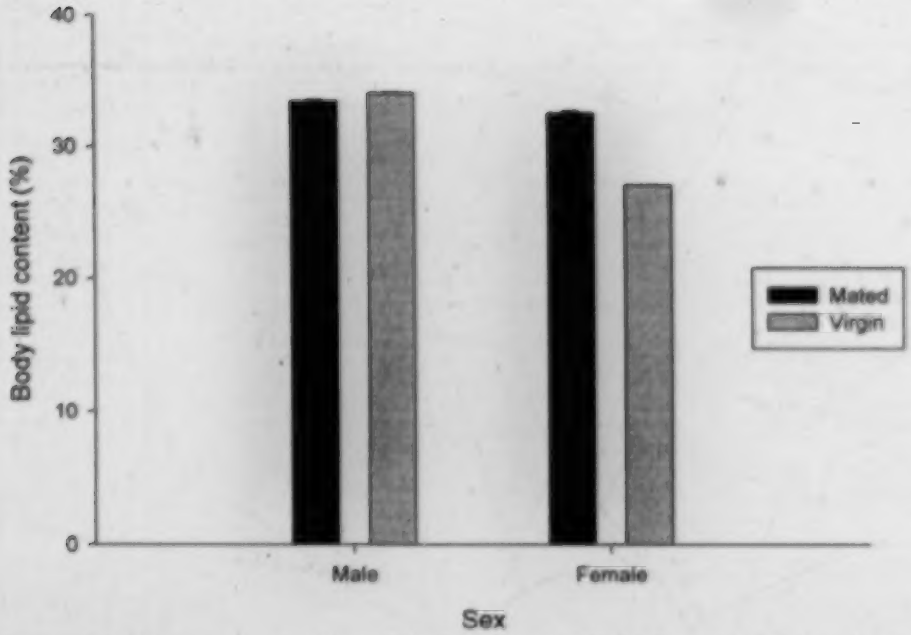

Figure 2.8. The percent body weight composed of lipid in male and female *C. conflictana* with different mating status. Lipid was measured using a soxhlet apparatus after moths died naturally. Bars represent standard error, $n_{male*mated}$= 100, $n_{male*virgin}$= 320, $n_{female*mated}$=92, $n_{female*virgin}$= 318. Lipid content values are adjusted to account for the covariate effects. Mated moths had a significantly higher lipid content than virgins (Stepwise GLM, $r^2 = 0.20$, $F_{1,821} = 8.97$, $p_{mating\ status} = 0.003$). Males had a significantly higher percent lipid content than females (Stepwise GLM, $r^2 = 0.20$, $F_{1,821} = 39.04$, $p_{sex} < 0.0005$).

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CHAPTER 3: A trade-off between flight and fecundity in *Choristoneura conflictana* (Walker) (Lepidoptera: Tortricidae)

INTRODUCTION

Movement is advantageous to an individual when it results in greater fitness benefits than remaining stationary (Bowler and Benton 2005). This dispersal may be long distance migration or spatially brief movement for the purposes of foraging (Loxdale and Lushai 1999; Bowler and Benton 2005). Dispersal varies substantially among species, between sexes and with development (Otronen and Hanski 1983). This is largely due to resource availability to a species or individual insect at a given time or place. In many insects, female reproductive investment is limited by energy reserves also used for flight dispersal (Wheeler 1996; Hanski *et al.* 2006) and can represent a major life-history trade-off (Johnson 1969; Stearns 1992).

Resources available to reproductively-active adult females can be determined genetically (Honěk 1993; Wedell and Karlsson 2003), or gained through feeding in the larval (Rivero *et al.* 2001; Awmack and Leather 2002; Nealis and Régnière 2004) or adult (Wheeler 1996; Oberhauser 1997) stages. Both exogenous and endogenous factors will therefore influence resource availability and allocation and may be particularly important in species that undergo population cycles, including many forest Lepidoptera that are under resource stress at high densities. Exogenous factors such as habitat availability and quality (Loxdale and Lushai 1999; Ims and Hjermann 2001) and mate availability (Leather 1988; Honěk 1993) affect the tendency of insects to disperse and the distance that they will travel based on the level of suitability of their current habitat, and the distance away from suitable conditions (Muller-Landau *et al.* 2003). Endogenous factors such as mating status (Outram 1971; Adams 2000) and time of mating (Evenden *et al.* 2006) alter the resources that a female may allocate toward dispersal, based on energy trade-offs with reproduction (Yanagi and Miyatake 2003; Elkin and Reid 2005). Additionally, female size (Jiménez-Pérez and Wang 2004) and paternal contributions (Wedell and Karlsson 2003;

Czesak and Fox 2003) interact to either increase or decrease energy allocation to dispersal (Shirai 1995), longevity (Ellers *et al.* 2000) and reproduction in insects, depending on availability of resources (Boggs 1992).

Insect species that undergo population cycles experience extreme variation in density that would make plasticity in life history strategies (Stearns 1992) adaptive. In such situations, changes in resource allocation by females can influence population dynamics in subsequent generations (Benton *et al.* 2005) and further exacerbate conditions that affect life history strategies of their offspring. At high densities, adult females should disperse (Doak 2000; Nealis and Régnière 2004; Bowler & Benton 2005) to find appropriate oviposition sites or feeding sites for their offspring. Inter-habitat dispersal requires high levels of investment in flight muscle development (Zera and Denno 1997; Marden 2000) and flight (Shirai 1995; Zhao & Zera 2002), and detrimentally affects egg production in some insects (Shirai 2006; Gu *et al.* 2006). Alternatively, dispersal in some insects is obligatory (Rudinsky 1962) and can be a prerequisite to egg production.

Here I test whether flight in adult females of *Choristoneura conflictana* (Walker) (Lepidoptera: Tortricidae) influences subsequent reproductive investment as measured by both potential and realized life-time fecundity. *C. conflictana* is a lepidopterous defoliator of trembling aspen, *Populus tremuloides* Michx. and is found throughout the range of its host in North America (Prentice 1955; Cerezke 1992). Populations of *C. conflictana* undergo cycles in which densities can increase suddenly and collapse quickly (Cerezke 1992). Therefore, density-dependent effects will be in constant flux and females need to rapidly respond through changes in resource allocation to maximize their fitness. Female size and disease status, two density-dependent factors, influence female reproductive output in this species (Evenden *et al* 2006), but it is not known if these factors influence resource allocation to flight, reproduction or longevity. My experiment is designed to determine if dispersal impacts resource allocation to reproduction differently in mated and virgin individuals. As female *C. conflictana* are winged and actively disperse under natural conditions (Prentice

1955), I can test if flight influences reproductive potential and determine if trade-offs in resource allocation exist in this cyclical forest pest.

METHODS

INSECT COLLECTION AND REARING

Moths were collected as late instar larvae and pupae from *P. tremuloides* stands near Priddis, AB (50.9° N, 114.3° W). Larvae were supplied with fresh aspen leaves until pupation. Pupae were separated by sex (Prentice 1955) and housed individually in 163 ml translucent plastic cups (Dixie®, Atlanta, GA). Female pupae were held under 16:8 light:dark cycle at 12-15 °C to spread out the eclosion period and provide a continuous supply of adult females for experimentation. Pupae were examined daily and newly eclosed moths were supplied with 10% (v:v) sugar water *ad libitum* through a cotton wick until experimentation.

FLIGHT DISPERSAL

Flight mill

Female moths were flown by tethering individuals to a fixed-arm (30cm) computer-linked flight mill (see Chapter 2 for details). Each rotation of the mill arm was registered by a small infra-red transmitter/receiver situated on the mill casing that sent a signal to the attached computer (Maxdigital, 250MB). A computer program (Flysumm, 2000 and 2006) written especially for use with these mills recorded each revolution (94 cm) as it occurred. Up to eight mills were run simultaneously on each day of experimentation depending on moth availability.

Flight assay

On each day of experimentation, sixteen (4-20 h-old) adult females were removed from rearing conditions 1 h prior to the initiation of the flight assay. Females were randomly divided into two groups of eight females. One group of females received the flight treatment and the other group was handled in the same manner described below for flown females but females were not attached to tethers and were not given the opportunity to fly. Females were chilled for ≤ 5 minutes at 4 °C and weighed to the nearest 0.01 mg. Moths were positioned with

soft forceps and pronotum scales were removed using a paintbrush (No. 1 artist's brush, Colours Classic). A piece of 1 mm (diam.) aluminum wire with a 2 mm (diam.) loop at one end and a 2 cm straight portion of wire perpendicular to the loop was used to tether each moth. The loop of each tether was dipped in contact cement (Lepage's® Pres-Tite Contact Cement, Brampton, ON) and held adjacent to the scale-free pronotum of each female for 20-30 seconds until the connection was secure. When all eight moths were tethered, they were transferred to a large, walk-in incubator that housed the flight mills. Each tether was attached to the distal end of the arm of one mill with a hollow piece of wire insulation at ~ 100 ° with the mill arm. Flights were initiated by 1-2 tarsal stimulations with a glass slide after which the moths remained untouched throughout the remainder of the 12 h flight assay. Assays were initiated 0.5 h prior to the onset of the scotophase and continued to 3.5 h into the photophase. At the end of each 12 h flight period, females were separated carefully from their tethers, weighed to 0.01 mg and transferred to a wax-lined 473 ml cardboard container fitted with a transparent plastic lid (Solo®, Chicago, IL) and provided with a source of 10% sugar water.

Reproductive Investment

Following flight, four of the eight females from each dispersal treatment (flown or not flown) group were given the opportunity to mate with a 2-4 day-old virgin male. Individual male moths were introduced into the cardboard containers housing females and pairs were provided with a 10% sugar solution and held under 16L:8D, at 23 °C until the female died. Containers housing moth pairs were positioned 40 cm underneath a webcam (Samsung Digimax 50, Concord, ON) that recorded images of the containers at 15 minute intervals using software (Webcam monitor 3.6, Plainview, NY). Images were recorded throughout the first 24 h period following pairing to record which moth pairs mated, when mating was initiated and how long pairs remained *in copula*. The area was illuminated using a red light (30 lux) during the dark period, and overhead fluorescent white light (268 lux) during the light period. The remaining females in each treatment group were held under the same conditions but remained

unpaired until the female died and were not photographed. The wax lining of the cardboard container served as an oviposition substrate.

Females in each treatment group were checked daily and female lifespan was recorded upon death. Dead females were frozen until dissection to count the number of chorionated eggs that remained in ovaries at death. The number of fertile and infertile eggs deposited on the waxy oviposition substrate over the females lifetime was counted using a dissecting microscope (60 x magnification). Potential lifetime fecundity was calculated by adding the number of fertile and infertile eggs laid by each female to the number of chorionated eggs remaining in the ovaries at death. Lifetime realized fecundity was calculated as the number of fertile and infertile eggs laid throughout each female's life.

In addition to the mass of the insect recorded before and after flight treatments, the area of the right forewing was measured as a metric of female size. At the end of the experiment, the right forewing was carefully removed from the female at the thorax and mounted using Elmer's Glue Stick on white paper. Wings were imaged using a computer scanner (Hewlett-Packard Scanjet 4070 Photosmart, Palo Alto, CA) and the area of each wing was calculated using a digital area measurement program (ImageJ, Version 1.37, 2006).

ANALYSES

The hypotheses that flight would impact the time of initiation and duration of mating were tested with two ANOVAs. The independent variables tested were female size and flight status, and the dependent variables were hour of the initiation of mating, and duration of copulation, respectively. The proportion of paired females that mated in each flight treatment group were compared with a Chi-square test.

To test the hypothesis that flight dispersal of adult female *C. conflictana* influences subsequent reproductive investment I used two separate Stepwise General Linear Model analyses (SYSTAT®12, 2007), one with potential fecundity and one with realized fecundity as the dependent variable, respectively. The independent variables in the model were flight treatment and mating status as

categorical variables, and longevity and pre-flight weight were treated as covariate factors. Each of the categorical variables were included as interaction terms with each of the covariate factors. Non-significant factors were removed from the model automatically in a step-wise manner until the most parsimonious model remained (SYSTAT®12, 2007). Individual pairwise comparisons were made using Tukey HSD (SYSTAT®12, 2007) on significant categorical factors generated from each model.

To determine if the distance flown or mating status impacted reproductive investment in females, two additional Stepwise General Linear Models were run using only females that had been subjected to the flight treatment. The total distance flown (x) over the 12 hour flight period was transformed to log(x) + 1 to improve normality. The dependent variables remained as potential and realized fecundity and independent variables included mating status, adult longevity, size, distance flown and proportion of total weight lost during flight. Interactions between variables of mating status and distance flown were also modeled with adult longevity, pre-flight weight, and proportion of total weight lost.

RESULTS

Flight Dispersal

There was no effect of flight treatment on the time in the scotophase that mating was initiated (ANOVA, $F_{1,60}$=0.220, p = 0.641). Moths initiated mating an average of 3.9 h (+/- 0.32 SE) into the scotophase. There was also no effect of mating on duration of copulation (ANOVA, $F_{1,60}$ = 1.671, p = 0.220). Moths mated for an average of 11.1 h (+/- 0.79 SE). Flight did not influence the propensity of females to mate as equal proportions of females from each flight treatment group mated when presented with a male (Chi square, $\chi^2 = 0$, p = 1.00). Of 51 flown females that were paired, 55% mated, and 45% did not, and of 92 paired females that did not fly, 48% mated and 52% did not.

Female lifetime potential fecundity was significantly affected by an interaction between flight treatment and mating status ($F_{1,259} = 4.68$, $p_{mating\ status*flight}$=0.031). When the effect of pre-flight weight was accounted for, lifetime

potential fecundity declined as a result of flight dispersal in virgin females but increased in mated females (Figure 3.1). There was a significant interaction between flight treatment and pre-flight weight with respect to lifetime realized fecundity ($F_{1,263}$= 4.74, $p_{flown*weight}$ = 0.03). Overall, females that were flown laid fewer eggs than females that did not fly (Figure 3.2). The distance flown by females did not directly affect their lifetime potential fecundity, but egg production was significantly affected by the proportion of weight lost over the flight period ($F_{1,93}$=19.19, $p_{weight\ loss}$<0.0001). Females that lost more weight, regardless of their mating status, produced fewer eggs (Figure 3.3A).

Pre-flight weight significantly influenced lifetime potential fecundity in flown females ($F_{1,93}$ = 10.82, p_{weight}=0.001), suggesting that female mas is largely due to egg load. There was a trend toward an interaction between distance flown and pre-flight weight ($F_{1,93}$=3.49, $p_{logdistance*weight}$=0.065) affecting egg production. This interaction may be weak due to the large variation in flight capability, but does show that females that disperse long distances produce fewer eggs than females that fly short distances.

The prediction of female lifetime realized fecundity was also strongly affected by weight lost over the flight period ($F_{1,98}$=7.57, $p_{weight\ loss}$=0.007). Increased weight loss as a result of flight resulted in fewer eggs laid by females (Figure 3.3B). Among flown moths, heavier females laid more eggs than lighter females ($F_{1,98}$=8.40, p_{weight}=0.005).

Female size and mating status

Pre-flight weight strongly affected both lifetime potential and realized fecundity of females but did so differently for mated and virgin females. There was a significant pre-flight weight by mating status interaction ($F_{1,259}$ = 8.42, $p_{mating\ status*weight}$ = 0.004) on lifetime potential fecundity. Large, virgin females produced more eggs than small virgins. In contrast, large, mated females produced fewer eggs than small, mated females (Figure 3.4). Mating status was a significant main factor in predicting lifetime realized fecundity ($F_{1,263}$=7.65, $p_{mating\ status}$=0.006) (Figure 3.5), as was pre-flight weight ($F_{1,263}$=33.07, p_{weight}<0.0001)

(Figure 3.6). These results indicate that mated females lay more eggs than virgins, and heavier females lay more eggs than lighter females.

Longevity

Female longevity significantly predicted lifetime potential fecundity only in an interaction term with mating status. There was a significant interaction between mating status and longevity ($F_{1,259} = 6.35$, $p_{\text{mating status*longevity}} = 0.012$) (Figure 3.7A) that showed longer-lived virgin females produced more eggs than short-lived virgins, but longer-lived mated females produced fewer eggs than shorter-lived, mated females. Longevity was a significant main effect influencing lifetime realized fecundity ($F_{1,263}=6.48$, $p_{\text{longevity}}=0.01$) (Figure 3.7B). Overall, females that lived longer laid significantly more eggs than those that died young. There was a marginally insignificant interaction between mating status and longevity ($F_{1,263}= 3.60$, $p_{\text{mating status*longevity}}=0.06$) on eggs laid, suggesting that mated moths laid more eggs earlier than virgin moths.

DISCUSSION

Mating status

Despite reports that insects that undergo regular population cycles generally do not disperse great distances (Price 1994; Javoiš *et al.* 2005), *Choristoneura fumiferana* (Clemens) (Lepidoptera:Tortricidae) is reported to leave densely populated sites by flight (Sanders and Lucuik 1975; Harvey 1977; Greenbank *et al.* 1980). Flight dispersal is essential for insect species that experience extreme differences in habitat due to cyclical population dynamics or poor resource availability (Otronen and Hanski 1983; Coll and Yuval 2004; Bowler and Benton 2005). The energetic investment in dispersal is known to affect other life processes, such as reproduction or longevity, in a resource allocation trade-off. Here, I show a significant interaction between flight and mating status in *C. conflictana* females in which flight positively affects potential fecundity in subsequently mated females, and negatively affects potential fecundity of virgin females. This supports the assumption that mating increases egg production

(Outram 1971; Loher *et al.* 1987; Wheeler 1996; Adams 2000), even when some energy has been used for dispersal.

Murata and Tojo (2002) suggest that the main sources for flight energy in insects are the unsaturated fatty acids found in triacylglycerol. Fatty acids not used during flight are transferred to the ovaries of females post-flight (Murata and Tojo 2002). If this is the case in *C. conflictana*, resources transferred post flight may result in the heightened fecundity I observed in flown and subsequently mated females in this study. In addition, females may potentially receive male-donated gifts during mating (Smedley and Eisner 1996; Czesak and Fox 2003). These male-derived resources are not present in virgin females and may account for lower egg production in the flown, virgin females I tested here.

Oviposition

Theory predicts that females of species that undergo population cycles should be less choosy about oviposition host and site than species that do not reach epidemic densities (Price 1994). However, other *Choristoneura* spp. readily discriminate between oviposition substrates, especially after mating (Wallace 2004). This may explain why there is a significant interaction between flight status and mating status that affects potential fecundity, but not realized fecundity in *C. conflictana*. Flight resulted in increased potential fecundity in mated females, however, there was no such relationship for the number of eggs laid by flown, mated females. Mated females may have lacked cues associated with natural oviposition substrates in this study and therefore did not oviposit their full egg compliment. Increased fecundity of flown, mated moths might be an important reproductive strategy for insects that eclose in a habitat that is not conducive to offspring survival (Loxdale and Lushai 1999; Ishiguri and Shirai 2004). Small, artificially starved *Choristoneura fumiferana* (Clem.) (Lepidoptera:Tortricidae) females fly prior to oviposition (Sanders and Lucuik 1975). At high population densities of *C. conflictana*, a lack of primary host material (*P. tremuloides*) can force larvae to consume less suitable host material, such as *Betula papyrifera* Marsh., *Salix* spp., and *Populus balsamifera* L.

(Beckwith 1970). High densities of larvae can also result in early pupation and smaller pupal size (Goulson and Cory 1995; Klemola *et al.* 2004). Both of these situations may prompt *C. conflictana* to disperse when under food stress in defoliated areas.

Female size

In addition to the effects of flight itself on fecundity, I tested if the total distance flown by each female affected potential and realized fecundity. Interaction between total distance flown and pre-flight weight explained some variation ($p = 0.065$) in the total eggs produced. This indicates that energy expended on flight may have different physiological effects on moths of different sizes. This is similar to observations on female *Plutella xylostella* (Lepidoptera: Plutellidae), in which flight impacts subsequent fecundity of small females to a greater extent than large females (Shirai 1995). Leather (1984) found that weight loss affects lifetime egg production more than body size. I saw a negative relationship between female weight lost over the flight period and both lifetime potential and realized fecundity. This indicates that the amount of energy used during flight directly affects the number of eggs produced and laid by female *C. conflictana*. It is likely that weight lost during flight is a good measure of expended resources because of endogenous differences in flight musculature (Marden 2000), resource allocation (Boggs and Ross 1993) , or wing loading (Coll and Yuval 2004) among individuals. Consequently, distance flown by individual females may be a poor indication of actual energy invested.

Body size affects realized fecundity in insects indirectly through impacts on longevity (Leather 1988; Honěk 1993) and can have lasting effects on the population (Benton *et al.* 2005). In my experiment, female weight affected potential and realized fecundity as a main effect and in interactions with other factors in the model. Body size may be particularly relevant in this system, because of changes in density of *C. conflictana* populations. Feeding on suboptimal hosts in response to a high density population increases development time and may produce smaller insects (Wickman 1963; Beckwith 1970; Roff

1981; Goulson and Cory 1995; Klingenberg and Spence 1997). Herbivory-induced chemical defenses produced by *P. tremuloides* reduce food quality and affect size and survivorship of *C. conflictana* (Clausen *et al.* 1989). Alternatively, a shortage of food may force larvae to stop feeding and pupate early (Klemola *et al.* 2004). These factors combined may enhance the effect of size, and consequently the dispersal capability of natural *C. conflictana* populations.

There was a significant interaction between body weight and mating status of *C. confictana* that showed among mated females, small moths produce more eggs than large moths. The best strategy for small females may be to produce a large number of small eggs (Iyengar and Eisner 2002). Alternatively, small female *C. conflictana* and other *Choristoneura* species, may utilize less energy for flight or survival than large females (Sanders and Lucuik 1975; Roff 1991). Small females may use resources obtained from feeding or transferred from males (Wheeler 1996; Smedley and Eisner 1996) more efficiently than large females. In *C. fumiferana*, small individuals have a greater propensity for flight prior to egg laying (Sanders and Lucuik 1975). In my experiments (Chapter 2), mated female *C. conflictana* did fly farther than virgin females. However, because many females oviposited prior to the onset of the flight period, it cannot be established whether size had the same effect on flight of mated, egg-laden females as observed for *C. fumiferana.*

Longevity

As expected in a species that produces eggs continuously, long-lived *C. conflictana* females laid more eggs than short-lived individuals (Harvey 1977; Wiklund and Persson 1983). This was in contrast to the findings of Evenden *et al.* (2006) in which no relationship between realized fecundity and longevity was detected. Insects with continuous egg production are known to live longer lives than insects that eclose with their full egg complement (Jervis and Ferns 2004), indicating a trade-off in allocation of energy between reproduction and survival.

There was a significant interaction between mating status and longevity that affected potential fecundity of *C. conflictana.* Interestingly, virgin females

produce more eggs if they live longer, but among mated females, short-lived moths produce more eggs than long-lived moths. Egg production may be concentrated in the first few days post eclosion (Harvey 1977; Jervis *et al.* 2001; Jervis and Ferns 2004) and may adversely affect longevity. The costs of this early reproductive ouput may include the physical costs of mating (Yukilevich 2005). Early reproductive output is correlated with lower output later in life in *Lobesia botrana* Denis and Schiffermüller (Lepidoptera: Tortricidae) (Torres-Vila and Rodriguez-Molina 2002), which may be why the most productive *C. conflictana* had relatively short adult lifespans in my study. The first eggs are more costly to produce (Rosenheim 1999) and *C. fumiferana* produce large eggs first in a clutch to increase chances of their survival (Harvey 1977). Egg resorption has also been noted to occur in insects that continually produce eggs if the female does not mate or encounter suitable oviposition sites (Rosenheim 2000; Wallace *et al.* 2004), which would result in a reallocation of reproductive energy to survival in long-lived females.

Density

Mating status, flight treatment and flight distance all affected reproductive capacity of female *C. conflictana*. However, regardless of these differences, potential and realized fecundity consistently depended on female pre-flight weight. Although more regulated under laboratory conditions, this relationship between size and fecundity has the potential to be broadly plastic under natural conditions, where population densities of *C. conflictana* are largely dynamic. Under dense conditions, insect size can fluctuate due to food shortage (Klemola *et al.* 2004) or feeding on inferior hosts (Beckwith 1970). Differences in body size of both males and females, can affect subsequent generations of insects and their capabilities to mate, disperse, and produce and lay eggs (Sanders and Lucuik 1975; Czesak and Fox 2003; Benton *et al.* 2005).

Insect body size can enhance the trade-offs in resource allocation between flight and mating, mating and longevity, and the subsequent effects on fecundity. Due to the intense effect of exogenous factors on egg production and realized

fecundity, resource allocation in species that undergo population cycles is highly dependent on the interaction of habitat and environmental features with genetics and physiology. Assessment of all the factors that could potentially affect *C. conflictana* fecundity was beyond the scope of this study. A more natural set of experiments could better assess environmental factors that influence fecundity and further assess the causes of body size variation in this species.

Potential and realized fecundity in *C. conflictana* appear to be largely dependent on flight treatment when the effect of mating status is controlled. Fecundity is largely determined by the size of the female. These relationships indicate a trade-off between flight and fecundity, and suggest that increased resources associated with larger size allow for greater reproductive capacity. Longevity had more of an effect on realized than potential fecundity, which indicates that when resources are abundant, as in a laboratory situation, longevity imposes a time limitation on egg laying more so than egg production. This may be affected differently in a natural environment as resource competition could limit longevity, alter resource allocation, and change patterns of oviposition. The observed significant interactions of flight with pre-flight weight and flight with mating status to determine fecundity suggest detrimental effects of flight on insect fitness, and possibly lasting effects on population dynamics because of the resource use necessary for flight dispersal.

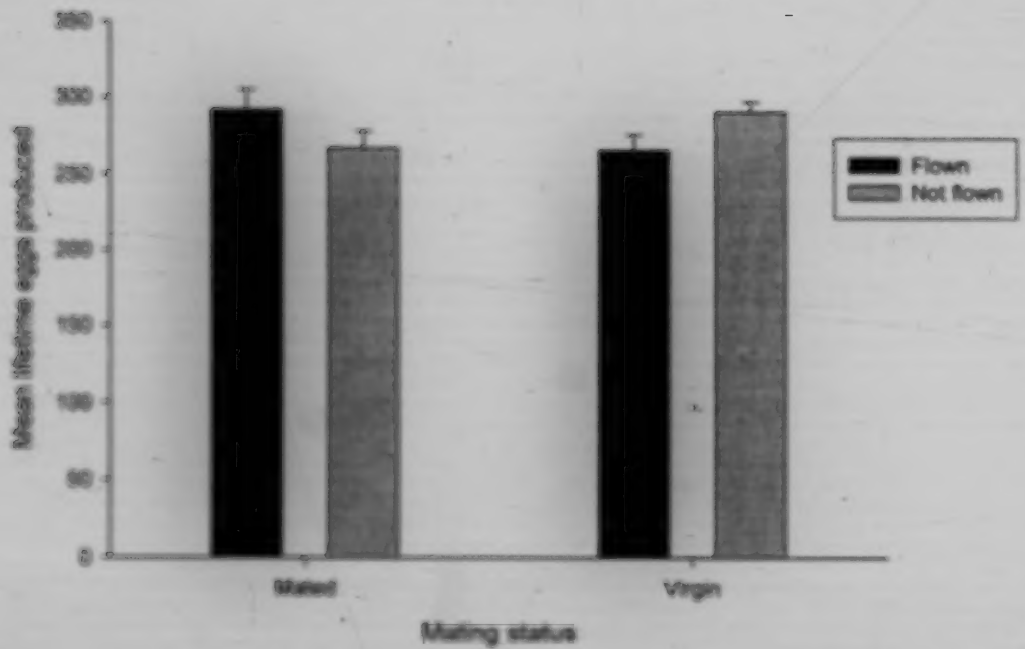


Figure 3.1. The mean lifetime potential fecundity of flown and not flown, mated and virgin females. There was a significant interaction between flight status and mating status to determine potential fecundity ($F_{1,239} = 4.68$, $p_{\text{mating status*flight}}=0.031$). Fecundity values are adjusted to account for the variation of other factors in the model. Bars represent standard error, $n_{\text{mated*flown}}= 21$, $n_{\text{mated*not flown}}= 42$, $n_{\text{virgin*flown}}= 60$, $n_{\text{virgin*not flown}}= 142$.

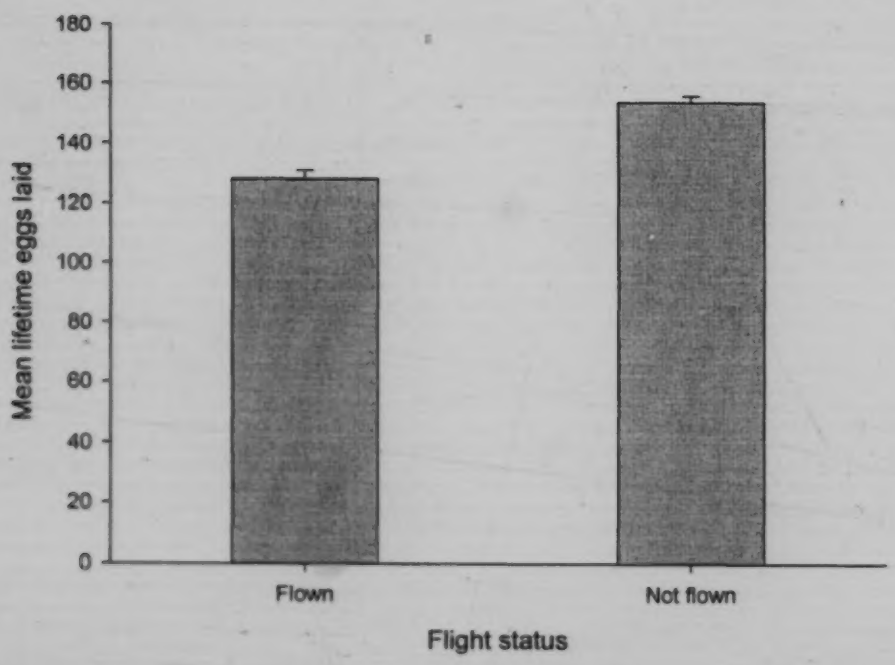
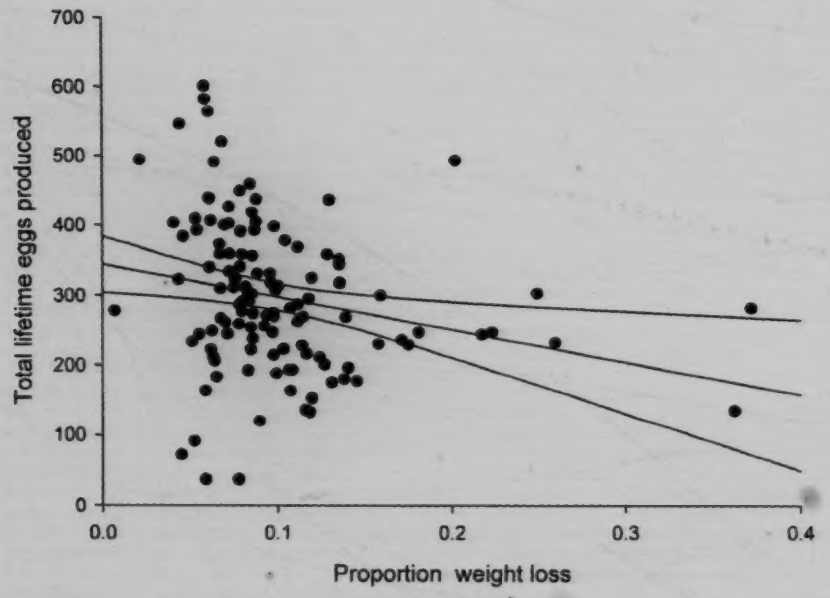


Figure 3.2. The mean lifetime realized fecundity of flown and unflown females. There was a significant interaction between flight status and pre-flight weight ($F_{1,263}$= 4.74, $p_{flown*weight}$ = 0.03) to affect realized fecundity. Fecundity values are adjusted to account for the variation of other factors in the model. Bars represent standard error, n_{flown}= 103 , $n_{not\ flown}$= 160.



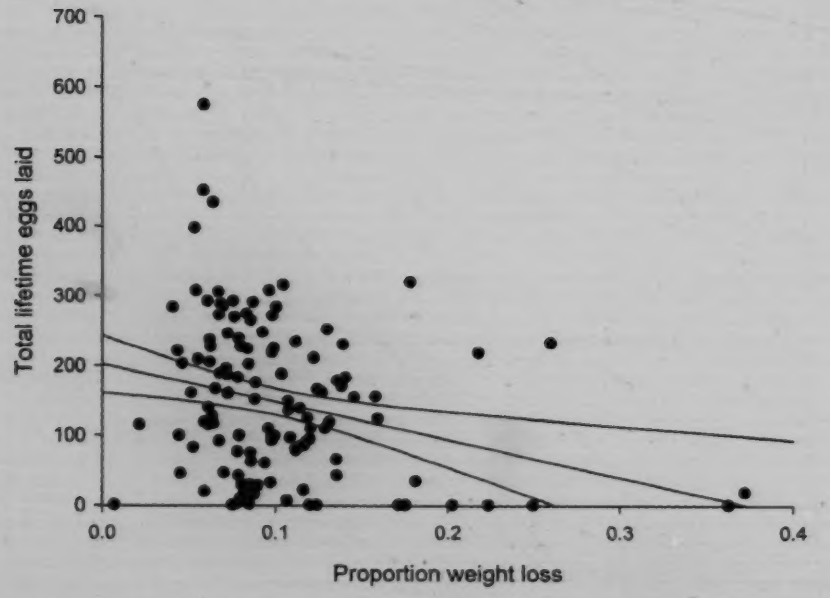


Figure 3.3. The relationship between proportion of weight lost by females during flight and A) lifetime potential fecundity ($F_{1,93}$=19.19, $p_{weight\ loss}$<0.0001, model r^2=0.48) and B) lifetime realized fecundity ($F_{1,98}$=7.57, $p_{weight\ loss}$=0.007, model r^2= 0.28) (B). The lines represent 95% confidence intervals.

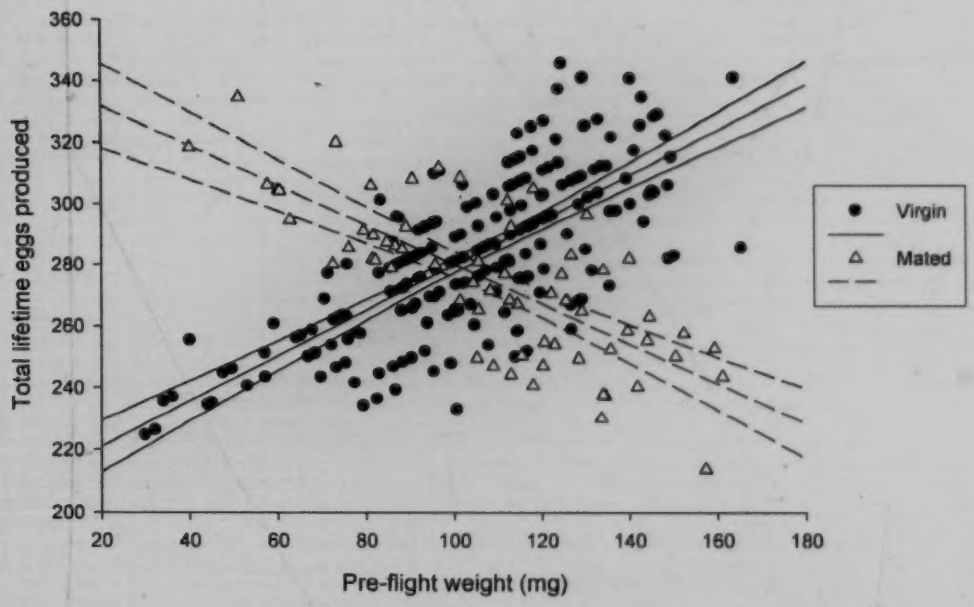


Figure 3.4. The relationship between mated and virgin female pre-flight weight and potential fecundity. There was a significant interaction between pre-flight weight and mating status in predicting potential fecundity ($F_{1, 259} = 8.42$, $p_{\text{mating status*weight}} = 0.004$, model $r^2 = 0.27$). Fecundity values are adjusted to account for variation of other factors in the model. Lines indicate the 95% confidence intervals.

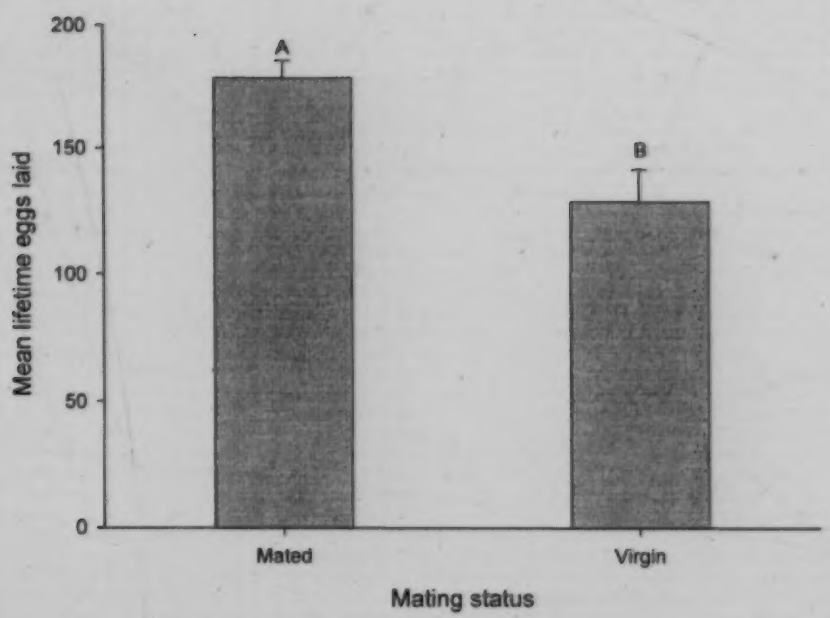


Figure 3.5. The difference in mean realized fecundity between mated and virgin females. Mated females laid significantly more eggs than virgin females ($F_{1,263}$=7.65, $p_{mating\ status}$=0.006). Fecundity values are adjusted to account for other factors in the model. Bars indicate standard error, n_{mated}= 63 , n_{virgin}= 202.

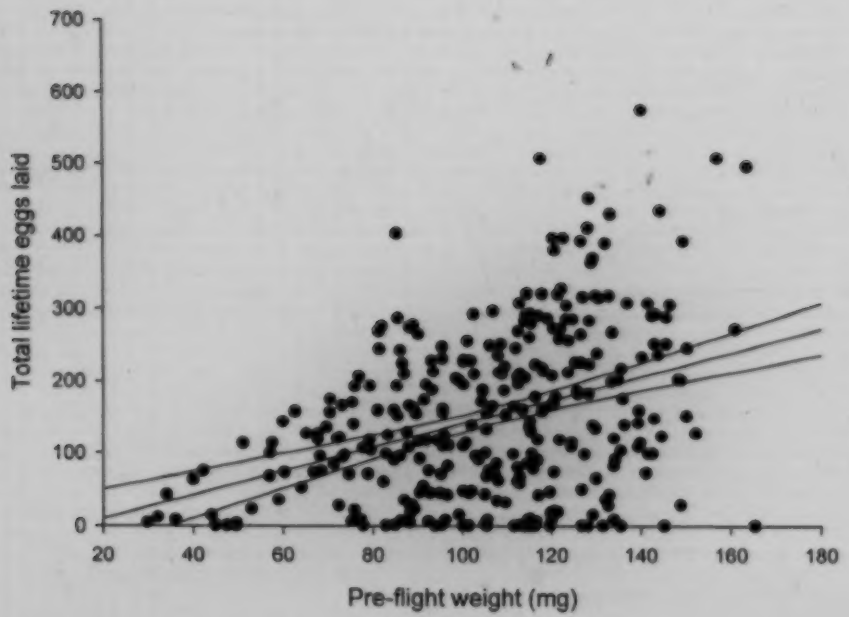
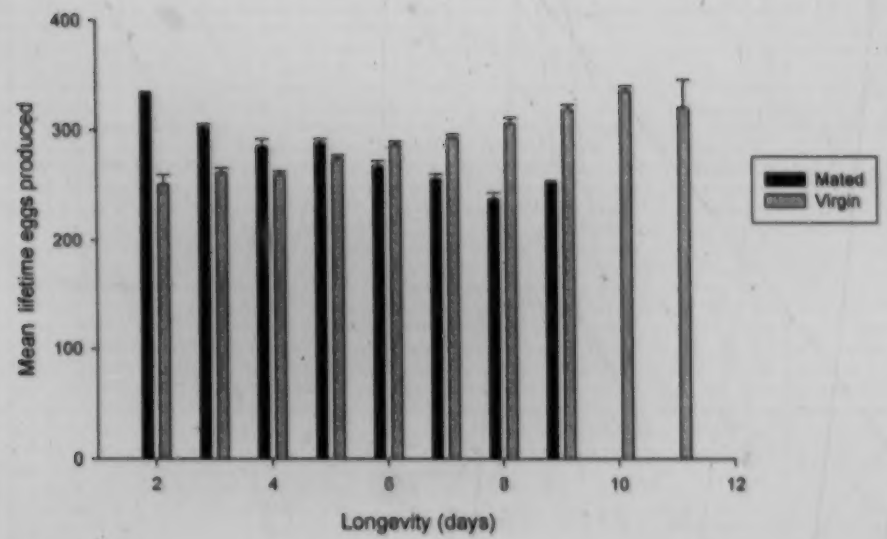


Figure 3.6. The relationship between pre-flight weight and realized fecundity in all females ($F_{1,263}$= 33.07, p_{weight}<0.0001). Lines represent 95% confidence intervals.



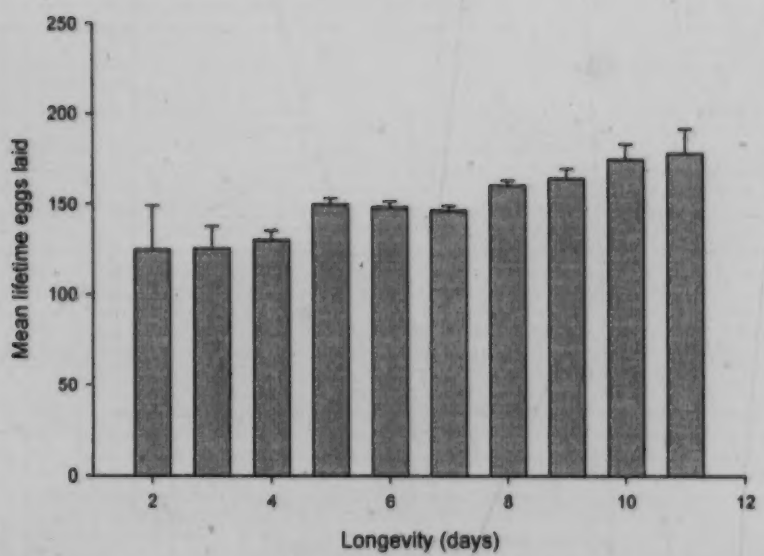


Figure 3.7. A) The interaction of longevity and mating status to determine eggs produced ($F_{1,259} = 6.35$, $p_{\text{mating status*longevity}} = 0.012$). B) The effect of female moth longevity on mean lifetime realized fecundity ($F_{1,263} = 6.48$, $p_{\text{longevity}} = 0.01$). Fecundity values are adjusted to account for other variables in the model. Bars represent standard error.

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CHAPTER 4: GENERAL CONCLUSION

Analysis of factors influencing dispersal capacity of forest Lepidoptera will help us to understand the cyclical nature of these populations. Here I have demonstrated that adult flight dispersal of *Choristoneura conflictana* is impacted by physiological factors such as age, size, lipid content, sex and mating status. I further show that two important trade-offs will impact *C. conflictana* fitness: 1) a trade-off between flight dispersal and realized fecundity; and 2) a trade-off between longevity and realized fecundity.

Distances flown by *C. conflictana* were highly variable, spanning from 0 – 5,000 m in males, and 0 – 26,000 m in females, over the 12-hour flight period permitted in all experiments. Despite this variability, pre-flight weight was a main factor that determined distance flown by moths, as well as potential and realized fecundity of females. Size is a trait of utmost importance for reproduction and survival in insects, and as such is often used as an intrinsic variable in fitness measures (Roff 1981). In Chapter 2, the distances flown by *C. conflictana* were dependent largely on moth size and the efficiency of energy utilization, which was measured by weight loss over the flight period and percent body lipid content. Size had an effect on potential and realized fecundity (Chapter 3), as did flight responses, mating status and adult longevity. Condition-dependent dispersal is influenced by both habitat and individual conditions, both of which can impact insect body size (Iino and Hijiemoto 2000). Lipid stores, egg carrying capacity, and wing size are all affected by genetics and physiology (Roff and Fairbairn 2001; Iyengar and Eisner 2002). Environmental conditions such as larval food availability and quality (Tisdale and Seppington 2001; Awmack and Leather 2002; Moreau *et al.* 2006; Tammaru 1998), ambient temperature (Jones *et al.* 1982; Sibley and Atkinson 1999) and competition and density (Dmitriewicz 1979; Wylie and Gottland 1998; Tammaru *et al.* 2002) also affect adult body size.

Increased fitness in insects is usually associated with larger size (Wylie and Gottland 1998; Tammaru *et al.* 2002). In this study, large *C. conflictana*

females consistently flew farther in the population reared from the laboratory colony (Chapter 2) but this relationship was not evident in the wild-collected population (Chapter 3). In the wild population, the individuals were much larger in size than those in the lab population (Appendix III). Large, wild *C. conflictana* females may have passed a crucial threshold for ideal wing loading, an important physical measure that determines dispersal capacity (Coll and Yuval 2004). In this way, large *C. conflictana* females may disperse further until a certain, prohibitive size is reached, at which point dispersal capacity decreases. Large female insects have high reproductive capacity (Gu *et al.* 2006), which can negatively affect survival and dispersal (Stearns 1992).

An important environmental influence on natural populations and size of individual *C. conflictana* is population density (as a result of its cyclical dynamics). This is an important aspect to consider, due to the significant effect of size on aspects of *C. conflictana* flight and fecundity demonstrated here. Many studies note differences in insect dispersal in response to both larval and adult crowding. For example, Hughes *et al.* (2001) showed adult *Grapholita molesta* were more likely to disperse if they experienced crowding as larvae. Small, starved *Choristoneura fumiferana* were more active than well-fed moths (Sanders and Lucuik 1975). Flight activity was also influenced by adult crowding in *Rhagoletis indifferens* Curran (Diptera: Tephritidae). Crowded individuals flew longer than individuals with abundant resources (Senger *et al.* 2007). Doak (2000) found low pupal masses of *Itame andersoni* (Swett) (Lepidoptera: Geometridae) in densely populated patches that eclosed into small moths with an increased propensity to disperse. *C. conflictana* are known to experience similar, crowded conditions (Prentice 1955; Cerezke 1992) and are likely to feed on alternative hosts, at high densities (Prentice 1955).

Density may also induce dispersal to obtain oviposition sites. *C. fumiferana* prefer to oviposit specifically on *Picea glauca* Moench (Grant and Langevin 1994) or conifer foliage in general (Grant *et al.* 2007). Female *C. fumiferana* also prefer to oviposit in locations with few conspecifics (Leyva *et al.*

2003). In Chapter 2, mated *C. conflictana* females flew farther than virgins. In Chapter 3, mated females laid more eggs than virgins. Mated *C. fumiferana* also disperse further than virgin females (Sanders and Lucuik 1975) in flight bioassays. In this way, population dynamics may strongly influence movement of adult females.

Weight loss during flight and body lipid content after flight, two different measures of energy use, predicted flight and fecundity in *C. conflictana*. In Chapter 2, a significant interaction between weight lost over the flight period by *C. conflictana* males and mating status determined flight distance. Weight loss during flight was also a significant factor when the effect of male age on flight capacity was tested. A significant interaction between percent body lipid content and sex predicted flight distance. In Chapter 3, proportion of weight lost significantly affected both potential and realized fecundity, in females that were flown on mills. The significance of these measures of energy expenditure further demonstrates the importance of both endogenous and exogenous factors on dispersal (Nylin and Gotthard 1998; Loxdale and Lushai 1999; Ims and Hjermann 2001). The high variability in fecundity and flight capacity also suggests genetic variability in these traits, as individuals respond differently to the experimental factors when exposed under similar conditions. These interactions would likely be further exacerbated in dense, outbreak populations in which individual plasticity would be influenced by the natural environment.

Due to the cyclical population dynamics displayed in natural populations of *C. conflictana*, understanding their movement and reproductive capacity is extremely important for understanding the spread of infestations and the initial cause of forest outbreaks. With a better understanding of defoliator dispersal and the effect that such energy expenditure has on the production of offspring, the impact of defoliators on economically profitable forests can be predictable and monitored. The increasing importance of *P. tremuloides* in forests and documentation of interactions between *C. conflictana* and other common aspen

defoliators (Cerezke 1992) necessitates further study into their dynamics, both within population and within the forest community.

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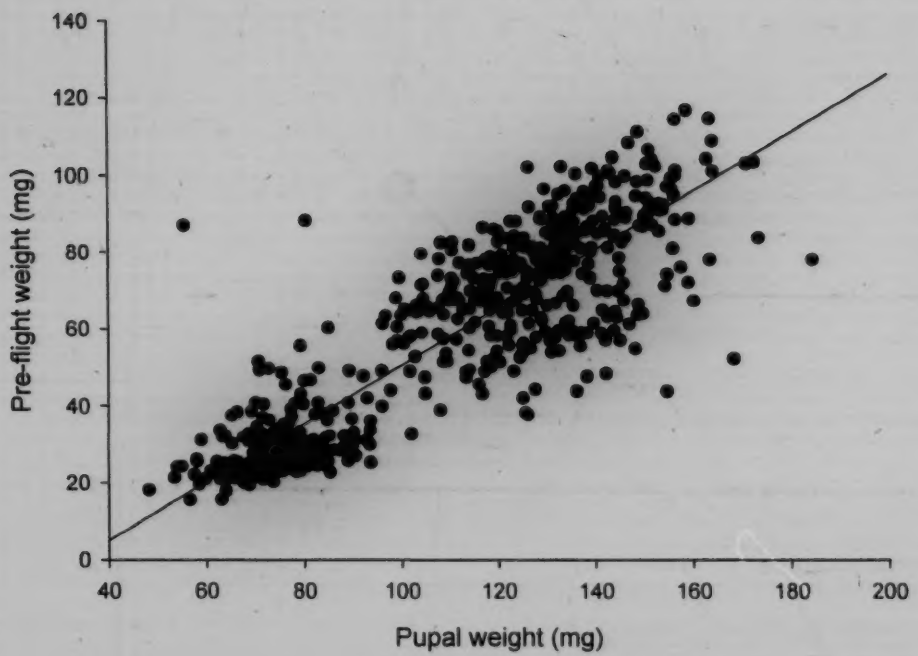
Appendix I

Spruce Budworm Diet used for rearing *C. conflictana*
BioServ, product #F9769B
1, 8th Street, Suite 1
Frenchtown, NJ 08825

1 L of diet:

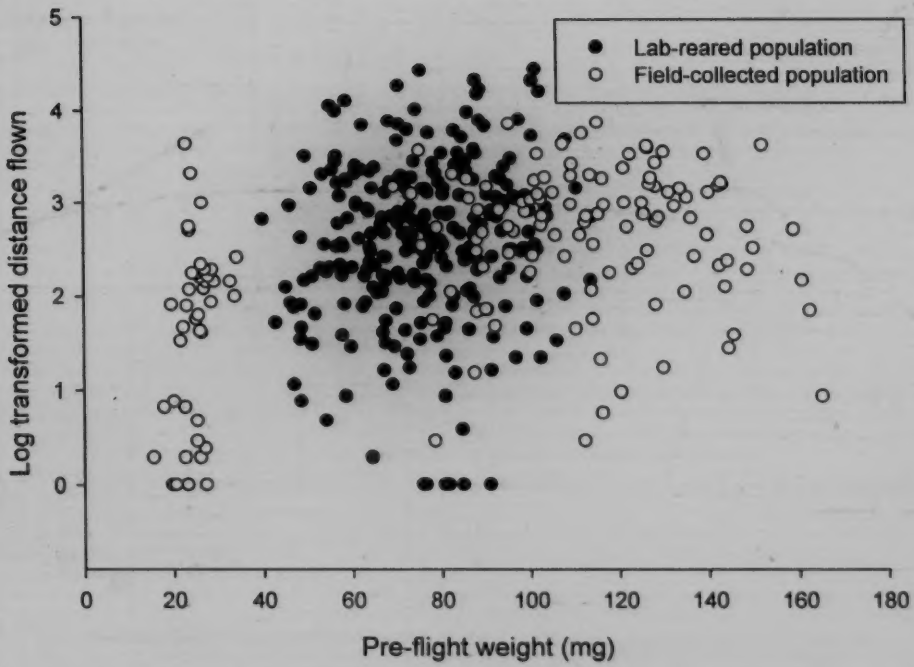
Agar	25.3 g
Dry mix	145.2 g
-Sucrose	
-Choline chloride	
-Ascorbic acid	
-Methyl paraben	
-Aureomycin (14.1% active)	
-Vitamin Mix, Lepidoptera #722 (BioServ)	
-Wheat germ, stabilized, 60 Mesh	
-Casein	
-Cellulose	
-Wesson salt mix	
-Raw linseed oil	
KOH Solution	5.1 g

Appendix II



The relationship between pupal weight (mg) measured on the first day of pupation and adult pre-flight weight (mg) of *Choristoneura conflictana* taken prior to flight (0 or 1 day post-eclosion) on computer-linked flight mills.

Appendix III



The relationship between pre-flight weight (mg) and log-transformed distance flown of lab-reared (Chapter 2) and field-collected (Chapter 3) populations of female *Choristoneura conflictana.*